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STUDIES ON THE HELMINTH FAUNA OF ALASKA. XXXI. OBSERVATIONS ON THE PROPAGATION OF THE LARVAL *ECHINOCOCCUS MULTILOCULARIS* LEUCKART, 1863, *IN VITRO*

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Larval reproduction in *Echinococcus multilocularis* Leuckart, 1863, takes place through the proliferation of exogenous secondary vesicles (Rausch, 1954). In some species of host this process appears to be continual, extending over a long period of time with little tendency for self-limitation. Consequently, the growth characteristics of this larval cestode are such that it would seem well suited to culture *in vitro*.

Beginning in November, 1955, the writers have attempted to propagate the larval *E. multilocularis* in tissue culture. This paper constitutes a preliminary report of the results of this work.

METHODS

Each of 4 experiments consisted of the inoculation of a series of culture tubes. The culture medium consisted essentially of 40% human ascitic fluid in Hank's basic salt solution. To this was added 100 units of penicillin and 100 μgm of streptomycin per ml. Phenol red is the indicator in Hank's solution. Some tubes were also inoculated with a malignant human epithelial cell (HeLa strain), which was allowed to become well established before the cestode tissue was introduced. In some cases, extract of vole embryo (*Microtus*) or human plasma was added.

Screw-cap tubes were used routinely, but Leighton tubes were also used in an attempt to follow better the development of individual vesicles. In an effort to immobilize groups of vesicles, perforated cellophane was placed in some of the tubes. The tubes were incubated at 35° C in a horizontal position without rotation.

Tissue of larval cestodes was collected aseptically from the livers of experimentally infected rodents. Three species of hosts were utilized: field vole, *Microtus pennsylvanicus* Ord; red-backed vole, *Clethrionomys rutilis* Pallas; brown lemming, *Lemmus sibiricus* Kerr.[1] After removal from the host, the larval tissue (in some cases with small quantities of hepatic cells) was finely divided with scissors. The resulting material was introduced by pipette into the culture tubes.

The medium was changed on an average of every 3 to 4 days, or whenever this was indicated by a drop in pH. In some instances, the pH was temporarily adjusted by the addition of isotonic sodium bicarbonate, or by the addition of 5% CO_2 in air.

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[1] The experimental animals, with the exception of the red-backed voles, were laboratory-reared.

From time to time, masses of larval vesicles were removed from the culture tubes for study. These were either stained with Semichon's acetic carmine and prepared as whole mounts, or were embedded by the paraffin method and sectioned. Sections were stained with hematoxylin-eosin.

In order to determine whether the larvae developed normally *in vitro,* fragments of tissue or small vesicles were removed from time to time and injected intraperitoneally into susceptible hosts. Such material was later recovered from the host for study.

RESULTS

Since the procedures followed differed for each, the 4 experiments are described separately.

Experiment I. Infective larvae of *E. multilocularis* were removed from the liver of a brown lemming, infected 171 days before. Five tubes were inoculated; 4 contained well established HeLa cells, and one contained only the basic medium. Intact scolices, freed from the enclosing vesicles, showed motility for a period of about 7 days. After this period, some showed evidence of degeneration; others, however, retained a normal appearance up to 60 days. Areas of hyaline tissue were noted on the 28th day in one of the tubes containing HeLa cells, and this was tentatively identified as larval tissue. Similar growth was noted on the walls of the other tubes during the next few days. By the 35th day, the HeLa cells appeared to be degenerating. No formation of vesicles was observed, and the experiment was discontinued on the 67th day.

Experiment II. The larval tissue was obtained from the liver of a red-backed vole infected 46 days previously; this material contained well developed scolices. Fifteen tubes were inoculated: 7 contained HeLa cells and vole embryo extract; 4 contained embryo extract without HeLa cells; 4 contained basic medium. Two tubes with HeLa cells were kept as controls.

On the 7th day after inoculation of the tubes, small areas of hyaline tissue were noted on the sides of a tube containing both HeLa cells and embryo extract. Some of this material, along with many HeLa cells, was scraped off the surface of the glass and withdrawn suspended in about 2 ml of medium. One ml of this suspension was injected intraperitoneally into 2 voles. One animal died early and was not examined. The second animal, when autopsied 45 days later, contained many free vesicles in the body cavity, and a mass of vesicles was attached to the abdominal wall at the site of injection.

Between the 10th and 16th days, hyaline growth was noted in the remainder of the inoculated tubes. This tissue was usually seen as minute plaques with clear centers. Proliferation of this tissue continued between the 16th and 26th days. On the 22nd day, vesicles were recognized in tubes containing HeLa cells and embryo extract; these were embedded in the layer of HeLa cells on the surface of the tubes. On the 29th day, the proliferation of larval vesicles was well advanced, with the best growth in those tubes having HeLa cells and embryo extract.

On the 30th day, some of the larger vesicles contained in their walls bodies identified as calcareous corpuscles (Fig. 1). The vesicles in the culture tubes were identical in structure and appearance with those seen at a similar stage of development *in vivo.* Transverse strands of tissue extended across the lumen of larger vesicles, possibly representing an early stage in the development of septa. Larval

vesicles were removed from a tube containing HeLa cells and embryo extract, and injected intraperitoneally into a vole. When the vole was killed 22 days later, 2 small vesicles, containing scolices, were found free in the body cavity. These closely resembled those seen in the tissue cultures on the 52nd day.

By the 34th day, the groups of vesicles were easily seen macroscopically. Single vesicles ranged up to 640 microns in diameter. Calcareous corpuscles and transverse strands of tissue were present in the larger vesicles. Vesicles were detached from the walls of 2 tubes which contained HeLa cells and embryo extract. From the first tube, a small quantity of tissue was fixed in 10% formalin. This tissue was divided; one half was prepared as a whole mount, and the other was sectioned. The study of this material disclosed numerous vesicles, ranging in size up to 1 mm,

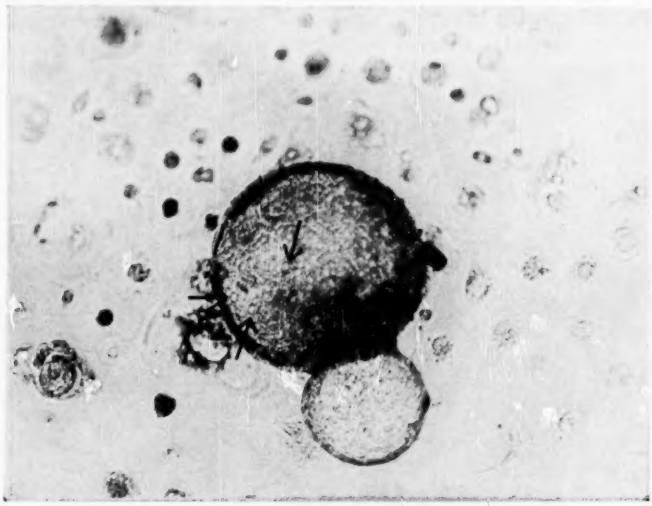

Figure 1. Early vesicles of *E. multilocularis* in tissue culture. The larger vesicle, measuring 360 microns in diameter, shows calcareous corpuscles, indicated by arrows.

embedded in a thick matrix of HeLa cells. The sub-germinal membrane was present, and measured about 5 microns in thickness. The germinal membrane was well developed and cellular. An immature but well developed scolex was observed in one of the sectioned vesicles.

From the first tube, above, tissue was injected intraperitoneally into 2 lemmings. One animal died within a few days, and was not examined. The second animal was examined 61 days later, and an abscess 15 mm in diameter was found at the site of injection. No evidence of larvae was noted. Two lemmings were also injected with material from the second tube, and examined 61 days later. In each a mass of vesicles was found in the subcutaneous tissue at the site of injection. Individual vesicles ranged in size up to 4 mm, and contained fully developed scolices.

By the 42nd day, masses of HeLa cells had surrounded the aggregations of vesicles, and the best growth of the larvae was seen in those tubes containing the cells and embryo extract. By this time masses of vesicles, with their surrounding

cells, had fallen away from the walls of the tubes and were free in the medium. Numerous separate vesicles were seen, these having become detached from the masses from which they had originated. Scolices were readily visible in larger vesicles.

Two lemmings were injected on the 48th day, and 2 red-backed voles were injected on the 52nd day. All were negative when examined from 44 to 47 days later.

On the 55th day, 2 vesicles contained 20 and 12 scolices, respectively. From the 56th to the 70th day, there was some degeneration of the HeLa cells, and this resulted in the freeing of more vesicles. In some tubes, the larvae had been completely overgrown by the cells, and appeared to be degenerating. On the 73rd day, it was noted that some vesicles had ruptured, and the scolices had fallen free in the medium. From the 73rd to the 120th days, the best vesicles were seen in the

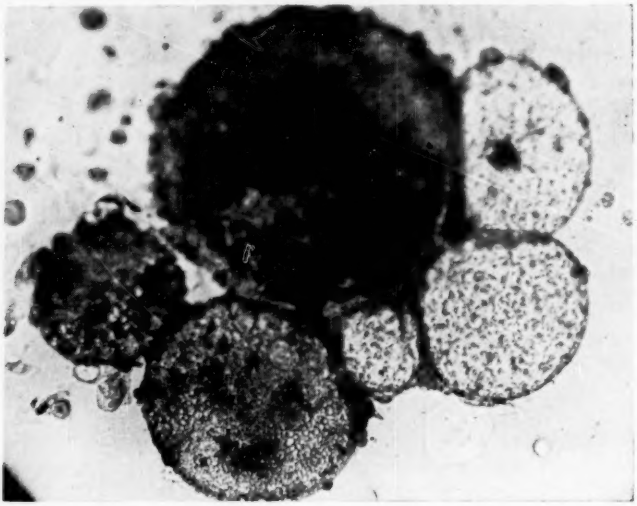

Figure 2. A group of vesicles of *E. multilocularis* produced *in vitro*, attached to the surface of the tube. The largest vesicle, measuring 500 microns in diameter, gave rise to the smaller vesicles, the most recent of which are characterized by very thin walls.

tubes containing only the basic medium. In some of the tubes containing HeLa cells, new areas of growth of the hyaline tissue were observed. The experiment was terminated on the 134th day.

Experiment III. Larvae were taken from the liver of a red-backed vole on the 10th day following infection. Forty tubes were inoculated, as follows: 13 Leighton tubes containing HeLa cells; 7 Leighton tubes containing HeLa cells and human plasma; 3 Leighton tubes and 3 screw-cap tubes containing basic medium with perforated cellophane; 3 Leighton tubes and 3 others containing basic medium and human plasma, with perforated cellophane; 8 tubes containing only basic medium. Four tubes containing HeLa cells were kept as controls.

Since many of the larval vesicles removed from the vole were very small, some were not ruptured when the tissue was divided with scissors. Such intact vesicles

were relatively thick-walled and were surrounded in part by hepatic cells. The proliferation of new vesicles was rapid, but these were readily distinguished from the original vesicles by their thin, fragile walls (Figs. 2 and 3).

On the third day, new vesicles ranging in diameter from 50 to 130 microns were observed, budding from the original vesicles. The latter were thin-walled and very fragile, sometimes rupturing when the medium was slightly agitated by rotation of the tube. Proliferation of new vesicles proceeded at about the same rate in all media. By the 12th day, vesicles measured up to 290 microns in diameter. Between the 17th and 24th day, many masses of vesicles no longer adhered to the walls of the tubes, making impossible continued observation of marked areas. Many of the tubes were discarded on the 19th day. On the 25th day, 8 single vesicles ranging in diameter from 500 to 1100 microns were isolated in tubes of basic medium. Seven days later these had grown very little, with a maximum increase in diameter of about 100 microns. Hepatic cells, introduced

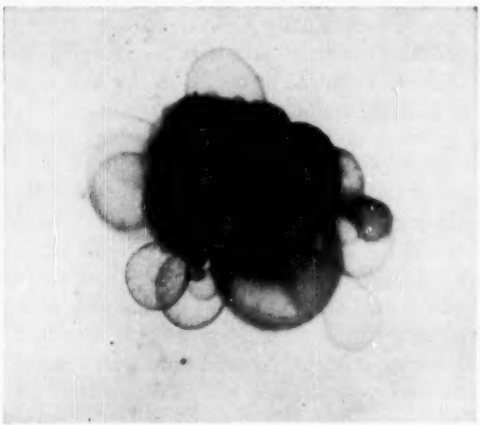

Figure 3. New vesicles of *E. multilocularis* arising from a vesicle inoculated intact. The latter is surrounded by host tissue. Total length of mass, 3 mm.

at the time of inoculation, were found among the Hela cells and persisted over 40 days. Proliferation of vesicles continued in the remaining tubes up to 40 days, but those in tubes containing HeLa cells were gradually surrounded and overgrown. Some tubes were observed as long as 63 days, after which the experiment was discontinued.

Experiment IV. Larval tissue was removed from the liver of a red-backed vole 37 days after infection. The tissue was washed in Hank's solution, transferred to a sterile Petri dish, and finely divided with scissors. Part of this tissue was added to a few ml of Hank's solution and filtered through sterile gauze. The filtrate was placed in the following tubes: 8 containing HeLa cells; 5 containing basic medium; 8 containing basic salt solution plus fresh ascitic fluid from a patient with carcinoma. Unfiltered tissue was placed in 6 tubes of basic medium, of which 3 contained perforated cellophane. On the 4th day, a heavy suspension of HeLa cells was added to half of all tubes not already containing them.

On the 7th day, vesicles from 180 to 400 microns in diameter were present in the tubes receiving the filtrate. Later growth of vesicles was poor, and observations were discontinued on the 24th day.

DISCUSSION

Previous attempts to culture larval cestodes of the genus *Echinococcus* have involved *E. granulosus* (Batsch, 1786). In the year 1900, Dévé (1926) tried unsuccessfully to maintain larval vesicles *in vitro*. Vesicles of human origin were placed in tubes containing human ascitic fluid; however, they had degenerated when examined 28 days later, apparently as a result of bacterial contamination. Dévé (1926) later was able to keep larval scolices *in vitro* for a period of 14 days, during which time some became vesicular. For this work, the contents of several vesicles of ovine origin were collected in sterile tubes. Into each tube was introduced 2 ml of clear hydatid fluid, and to this was added 0.5 to 1 ml of recently collected, unheated equine serum. The tubes were covered and incubated at 37° C. Samples of scolices were removed daily for examination. After the 4th day, some of the scolices had become "hydropic" and were becoming vesicular. By the 14th day, some of the scolices had doubled or tripled in size and were clearly vesicular. Dévé regarded these as being viable and in good condition.

Studies similar to those of Dévé were continued by Coutelen (1927a) who attempted to define some of the physical requirements *in vitro* of the larval *E. granulosus*. The larval cestodes were collected aseptically from the hosts, and placed in sterile containers. Various bacteriological media were tried, with negative results, but evagination of the scolices took place in sterile, unfiltered hydatid fluid. Some of these were becoming vesicular, but they did not survive long. Various media containing hydatid fluid and extracts of organs gave promising results, and scolices from the liver of a pig were maintained for 31 days in 2 media comprised of hydatid fluid with ascitic fluid and hydatid fluid with "extrait globulaire." By this time, the scolices were vesicular and had increased from 25 to 30 times their original volume. Coutelen was also able to keep nonsterile vesicles from the liver of a pig for as long as 12 days, and believed that they could have been maintained much longer *in vitro*, had they been bacteriologically sterile.

In his material, Coutelen noted that the scolices evaginated within a few hours, with a gradual increase in size within 3 or 4 days. This increase in size took place in both evaginated and unevaginated scolices. By the 10th day, some scolices had increased to about 10 times their original volume, and during this time became vesicular. Although there was some thickening of the cuticle, he did not observe the development of lamination. In some cultures, on about the second day, minute, thin-walled vesicles were seen protruding from some scolices. It was also believed that original capsules containing scolices ruptured, everted, and then repaired the break, resulting in a vesicle with the scolices protruding from the external wall. Coutelen noted that about 1 scolex in 50 became vesicular.

Coutelen (1927b) later studied serial sections of material which had been kept for 12 days in a mixture of hydatid fluid and unheated bovine serum. In this culture were found all intermediate stages between normal scolices and those which had become vesicular. The observation was confirmed that capsules containing scolices rupture, evert, and then regain their vesicular form by repair of the rup-

tured tissue. In this way, the thin layer of tissue formerly lining the vesicle covers the exterior surface. Coutelen made numerous observations on the development of vesicular scolices.

Dévé (1928) continued this work, being particularly concerned with the earlier failure to obtain actual lamination of the cuticle in vesicles derived from scolices. After several attempts, scolices were maintained for 43 days in a mixture of equal parts hydatid fluid and human ascitic fluid incubated at 37° C. Minute vesicles deriving from vesicular scolices were found to have relatively thick, laminated cuticles. Dévé also reported the successful culture of scolices in collodion sacs implanted in the peritoneal cavity of rabbits. After 79 days, vesicles measuring up to 5 mm in diameter were found upon removal of the collodion sacs.

Aside from the fact that the species of cestode was different, the experiments of Dévé and Coutelen differed basically from those reported herein; they were concerned mainly with the changes taking place in scolices kept *in vitro,* while we attempted to produce vesicles from undifferentiated germinal tissue. In our first experiment, we noted an increase in the size of some of the scolices kept *in vitro;* these did not become vesicular, however, and soon degenerated. Since the scolices are the end product of larval development, it does not appear that they would provide a good source of new vesicles. However, new attempts to culture *E. granulosus* would seem desirable.

As outlined above, the larval *E. multilocularis* proliferates and produces scolices *in vitro* about as rapidly as in suitable intermediate hosts such as *Microtus* and *Clethrionomys.* Such scolices are presumably infective, although a sufficient number has not yet been obtained to make practicable an attempt to infect a canine final host. The fact that larval tissue removed from the culture tubes proliferates and produces scolices when introduced into the peritoneal cavity of a natural intermediate host is another indication that *in vitro* conditions, as outlined above, are not detrimental.

In the present experiments, larval proliferation diminished after a certain maximum size was attained. The larval masses probably become so large that the medium surrounding them soon becomes unfavorable. The use of larger containers with a rotating mechanism to provide some circulation of the medium would probably be beneficial.

The importance of the HeLa cells is not yet clear. However, they provided a substrate on which the larval tissue became attached, and permitted the aggregations of vesicles developing from a common origin to remain intact. The first evidence of growth of the cestode tissue was almost always noted in tubes containing HeLa cells. It is possible that these cells in some way stimulated the proliferation of this tissue. In the tubes lacking HeLa cells, the vesicles usually were separate and free in the medium, without much tendency to form large aggregations. However, the HeLa cells in older cultures often enclosed the vesicles so completely that their effect was considered detrimental.

Observations of larvae *in vitro* confirm the earlier conclusion that proliferation takes place through the exogenous budding of new vesicles (Rausch, 1954). The fluid medium allows a retention by the vesicles of a spherical shape, while *in vivo* they are no doubt affected by the resistance of the host tissue in which they occur. It is of interest that endogenous budding of vesicles was not observed *in vitro,* and

there is so far no evidence that this takes place. Further study of the development of the larval *E. multilocularis in vitro* will facilitate an understanding of all aspects of its structure and growth.

It is believed that the availability of a larval cestode which is readily maintained *in vitro* may permit metabolic and other studies hitherto not practicable. It would also seem feasible to produce larval vesicles in quantities adequate for the production of antigens for use in the serological diagnosis of hydatid disease. This would have the advantage that the undesirable components of host tissue present in material obtained from infected animals would be lacking.

The larvae of *E. multilocularis* are capable of proliferating in a variety of mammals which are widely separated phylogenetically. In some host-species, the larvae develop normally and become infective; in others they are able to persist, but rarely produce infective scolices (Rausch and Schiller, 1956). This would seem to indicate an unusual capacity in this larval cestode to tolerate adverse conditions.

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RESEARCH NOTE

SARCOCYSTIS IN THE KINKAJOU*

Sarcosporidian infection has not been reported previously from the kinkajou (*Potos* sp.), a carnivorous mammal belonging to the family PROCYONIDAE. In January, 1953 an old male, with badly worn and chipped canine teeth, was shot from a corozal palm near the Rio Mandinga bridge, Panama Canal Zone, by Dr. R. H. Mitchell. At autopsy particular search was made for parasites, but the gastro-intestinal tract and blood smears were negative. Blocks of tissues cut from the vital organs and muscles were fixed in 10% formalin. Later, histological sections were prepared from these blocks and stained with haematoxylin and eosin at the Gorgas Hospital Laboratory. Study of this material revealed a sarcosporidian infection in the sections of striated muscle; none was found in the myocardium, or in the other organs. The cysts were common, but their presence was not suspected until after microscopic examination of the sections.

In longitudinal sections, the sarcocysts were broadly oval, measuring 123 to 216 microns long and 85 to 100 microns wide. They ranged from 63 to 200 microns in diameter in cross-sections of striated muscle, but the majority averaged about 77 microns. Each sarcocyst was bounded by a dense, well-defined, unstriated wall about 1 micron thick. The parasitized muscle fibers were enlarged to 2 or 3 times their normal diameter. The enclosed portion of the cyst was divided into compartments by a network of thin eosinophilic strands, the "Kammerung" of Miescher. These chambers were densely packed with banana-shaped sporozoites which were rounded at one end and pointed at the other. The sporozoites were 7.5 to 9 microns long and about 2 microns wide. The nucleus was usually located in the blunt end. There was no evidence of tissue reaction to the parasites in any of the sections examined.

This is the first report of an infection by *Sarcocystis* sp. in the kinkajou (*Potos* sp.).—MICHAEL J. TAKOS, *Department of Tropical Public Health, Harvard School of Public Health, Harvard University, Boston, Mass.* (*Aided in part by a grant from the United Fruit Company*)

OBSERVATIONS ON THE SURVIVAL *IN VITRO* OF BACTERIA-FREE ADULT COMMON LIVER FLUKES, *FASCIOLA HEPATICA* LINN., 1758*

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Although *Fasciola hepatica* Linn., 1758, has been studied *in vitro* previously, earlier investigators were dealing with contaminated, dying flukes. The previous work has been well reviewed by von Brand (1952) and Bueding and Most (1953). Only Stephenson (1947) was able to keep the fluke alive for more than a few hours, but he, too, had to contend with bacterial contamination.

Stephenson (1947) was able to keep the adult fluke alive for 75 hours in a borax-buffered saline containing 45 mM fructose per liter. The borax, in addition to being a buffer, acted as a bacteriostatic agent. Epps, Weiner and Bueding (1950) demonstrated a method for producing bacteria-free parasitic helminths when they used antibiotic solutions to produce bacteria-free *Ascaris lumbricoides*. Subsequent to the present investigation, Dawes (1954) reported that sterile *F. hepatica* adults could be obtained by cleaning the surface of the liver with absolute alcohol and then dissecting the livers in a "Dettol"-sprayed chamber. Dawes was able to maintain sterile flukes for up to 16 days in Hedon-Fleig solution.

The present paper describes a method for obtaining and maintaining bacteria-free flukes *in vitro*, the survival *in vitro* in the presence of simple carbohydrates, and the survival under aerobic and anaerobic conditions. It confirms Stephenson's (1947) data that this fluke apparently absorbs simple carbohydrates through its cuticle. An attempt is made to correlate the data presented with data obtained by other investigators in order to obtain a better understanding of the *in vivo* physiology of *F. hepatica*.

MATERIALS AND METHODS

The procedures outlined by Stephenson (1947) were used in the early stages of this investigation. Flukes, while still in the bile ducts, were brought from the packing plant into the laboratory where they were removed from the livers. After being washed with Stephenson's saline III (Table I), the flukes were placed in flasks containing this saline.

It was noticed that the longer the flukes remained in the bile ducts following the host's death, the shorter the time the flukes remained alive *in vitro*. Therefore, arrangements were made to permit removal of the parasites from the infected bovine livers immediately after the slaughter of the animals. All flukes used were from bovine sources. The livers were dissected on the killing floor; therefore, aseptic techniques could not be used, but gross contamination was held to a minimum. As

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flukes were expressed from the bile ducts, they were transferred to a flask containing sterile saline solution without glucose. Three salines were used (Table I).

TABLE 1.—*Basic salt solutions for* Fasciola hepatica *in vitro*

Compound	Grams per liter of distilled water		
	Tyrode's[1] (Cameron, 1950)	AB[2]	SS[3] (Stephenson, 1947)
NaCl	8.0	2.4	8.64
KCl	0.2	0.75	0.745
$CaCl_2$	0.2	0.55	0.11
$MgCl_2 \cdot 6H_2O$	0.1	0.2	
$NaH_2PO_4 \cdot H_2O$	0.05		
$Na_2HPO_4 \cdot 2H_2O$		2.7	
$NaHCO_3$	1.0	0.84	
Na_3 Citrate		2.9	
$Na_2B_4O_7 \cdot 10H_2O$			2.29
Glucose	1.0	3.6	5.4

[1] $NaHCO_3$ autoclaved separately and added after solution cooled.
[2] $NaHCO_3$ autoclaved separately; glucose autoclaved separately; both added after solutions had cooled.
[3] Glucose autoclaved separately and added after solutions had cooled.

The flukes were transported to the laboratory in the type of saline in which they were to be held later.

It was found that not more than 50 trematodes should be placed in 200 ml of solution because a greater number resulted in a high mortality of flukes during the 1 to 3 hours they were in this flask. This was probably due to metabolic wastes of the parasites and contaminants, plus breakdown products of blood, mucus and bile which were carried over with the flukes. The flasks were maintained at approximately 37° C while collecting at the packing plant and during transit to the laboratory, by keeping them surrounded by cotton saturated with warm water in a large thermos jug.

Routinely upon arrival at the laboratory, flukes were transferred to a basic saline solution containing either 25 mg aureomycin or 250 mg streptomycin and 100,000 units penicillin per 100 ml of solution. Following this transfer, aseptic techniques were used throughout. It was found that the streptomycin-penicillin solution rendered a greater percentage of flukes bacteria-free than did the aureomycin solution. Flukes were held in the antibiotic for 18 to 24 hours at $37 \pm 1°$ C and were maintained at this temperature for the balance of the experiment. If more than 25 flukes were held in 100 ml of antibiotic solution, an excessive mortality occurred, probably due to an excess of metabolic wastes.

After 18 hours in antibiotic solution, it was usually evident whether or not the flukes were bacteria-free. Contaminated flukes could usually be discerned by the appearance of small, blanched areas. Flasks containing flukes so marked were discarded.

Following the period in antibiotics, flukes were transferred to Petri dishes in which it was possible to check them for injuries and to select specimens of comparable size, color and vigor. These criteria were used for selecting pairs for further experimentation as no method was found for determining age of flukes except for the immature and senile individuals which were discarded.

Four-ounce oil sample bottles which could be sealed with thin-centered soft rubber stoppers were used to hold the flukes. This permitted the substitution of gases and the addition of nutrients by means of a hypodermic needle. Routinely only 1 fluke was placed in a bottle containing 50 ml of fluid.

At the beginning of the investigation, possible bacterial contamination was determined by drawing off a sample of fluid and placing it in thioglycollate broth or by making a thioglycollate stab. It soon became apparent that if the flask was contaminated, the solution in which the fluke was held rapidly became cloudy so that subculturing was unnecessary.

Autoclaved liver extract for this investigation was prepared by macerating cattle liver in a Waring blendor with an equal volume of distilled water, autoclaving to coagulate the protein, and then filtering. The filtrate was then bottled and autoclaved. It was used in proportion of 200 ml to 800 ml of basic saline.

To supply the heat-labile substances from liver, Injection Crude Liver-MRT (manufactured by Marvin R. Thompson, Inc., Stamford, Conn.) was dispensed in quantities of 10 ml per liter of solution. Other substrates substituted for glucose during this investigation were dispensed at 1 gram per liter of solution. Solutions were changed at weekly intervals, decanting the old fluid, leaving approximately 10 ml of solution still covering the fluke.

All flukes except where otherwise noted were maintained under aerobic conditions. Anaerobic conditions were established by evacuating the bottle containing the solution with an oil-vacuum pump for 3 minutes, tapping the bottle to help release dissolved gases, filling the bottle with nitrogen, shaking the bottle, reevacuating and then refilling with nitrogen. The stopper was removed to insert the trematode, restoppered and then the gaseous phase was swept out with nitrogen. Aero- was used instead of nitrogen.

In changing fluids during anaerobic experiments, the fluid which was first treated as above was gently poured in to prevent excessive mixture with air; the gaseous phase was swept out; the bottle was swirled vigorously to help replace absorbed gases; and then the gaseous phase was again swept out. It was not possible to evacuate bottles containing flukes as the absorbed gases in the parasites would expand, killing or injuring them.

To verify Stephenson's (1947) data on the absorption of carbohydrates through the cuticle, flukes were ligated with surgical silk immediately anterior to the ventral sucker. The silk was pre-tied in a friction knot and sterilized in Tyrode's solution. It was found that if flukes were placed on their dorsal surfaces in shallow fluid, they would extend their cephalic cones upwards; this facilitated slipping the wet loop of silk over the cone.

Prior to daily examination of the flukes, the bottles were swirled vigorously to disperse waste materials more evenly throughout the solution and to effect the exchange of gases.

Only flukes with no visible signs of food in their guts were used during this investigation.

Results

Maintenance in vitro *under aerobic conditions.*

The more important data of various trials to maintain this parasite *in vitro* are summarized in Table II.

It was found that if flukes were brought back to the laboratory while still in the bile ducts, they would survive in Stephenson's saline for up to 72 hours. When the flukes were removed at the packing plant, they survived *in vitro* for an addi-

TABLE II.—*Survival of* Fasciola hepatica in vitro *in various media under aerobic conditions*

		Number of Flukes Surviving																							
		In days																							
Soln	Compound	0	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	20	1	2	3	4
SS*	(Not sterile)	8	8	8	8	0																				
SS	(Not sterile)	3	3	3	3	2	0																			
SS		3	3	3	2	2	1	1	1	1	1	0														
AB		3	3	3	3	3	3	2	2	2	2	2	2	2	0											
Ty		11	11	11	11	11	11	11	11	10	9	5	3	3	3	3	2	2	2	1	1	1	1	1	1	0
Ty	ALE	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	0		
AB	ALE	14	14	13	13	10	8	8	8	5	4	4	3	3	2	2	2	2	2	2	2	2	2	2	2	0
AB	ALE + ICL	18	18	18	18	18	17	16	16	14	13	13	12	10	9	9	8	8	8	8	8	8	8	8	8	8**

* Flukes brought back to the laboratory while still in bile ducts.
** Experiment concluded at 30 days as flukes were moribund.
SS = Stephenson's saline ; AB = saline described in Table 1 ; Ty = Tyrode's solution ; ALE = autoclaved liver extract ; ICL = Injection Crude Liver.

tional 24 hours. When aureomycin was used to control bacterial contaminants, flukes survived for 9 days in Stephenson's saline.

When the substitution of 2.4 gm Na_2HPO_4 for the borax proved to be of no value, no further modification of this solution was attempted. A new solution, designated as "AB" in the tables was designed to approximate more closely the inorganic constituents of bile and was buffered at pH 8.5, which Stephenson (1947) had found to be best. Antibiotic-sterilized flukes survived for 12 days in this solution. Tyrode's solution permitted survival *in vitro* for up to 21 days, but the flukes were obviously dying after 1 week.

Flukes that had no glucose or other organic substances available in solution failed to survive 72 hours *in vitro*.

Neither the addition of 350 mg/liter of para aminobenzoic acid nor a slight modification of a combination of amino acids designated as Fischer V614 (Cameron, 1950) (cysteine HCl, 15 mg; glutathione, 10 mg; l-lysine HCl, 30 mg; l-histidine HCl, 10 mg; l-arginine HCl, 7 mg; d,l-valine, 28 mg; l-leucine, 18 mg; d,l-isoleucine, 20 mg; l-threonine, 12 mg; l-phenylalanine, 14 mg; l-tryptophane, 4 mg; d,l-methionine, 10 mg; and glutamine, 250 mg per liter) proved to be of any value.

The addition of 1.8 gm cholesterol per liter of solution did not prolong survival time, but did extend the period of normal motility and color for a week and a half. The addition of Injection Crude Liver to cholesterol had no apparent effect.

The addition of autoclaved liver extract extended the period of normal motility and color to 2 weeks and survival to 3 weeks. When Injection Crude Liver was added to solution AB containing autoclaved liver extract, the flukes remained in good condition for 3 weeks and survived up to 30 days, when the experiments were terminated because the flukes were moribund.

An attempt to maintain *F. hepatica* in tissue culture materials (chicken plasma, horse serum and beef embryo extract) met with failure. The plasma clots loosened after a few days, which made the transfer of liquids almost impossible. Although the flukes were extremely active for the first few days, they failed rapidly, and the experiments were concluded at a week's time. These poor results could have been caused by the use of only 10 ml of liquid in each flask. While this proved to be sufficient fluid for opisthorchids (Hoeppli and Chu, 1937) it might not have been enough to disperse the waste materials of *F. hepatica*. Unfortunately during this investigation it was not possible to use these materials in large quantities.

Therefore, a solution of 200 ml autoclaved liver extract, 10 ml Injection Crude Liver, and 800 ml of AB solution was the best medium found for the axenic maintenance of *F. hepatica in vitro* during this investigation although it is still obviously lacking some essential factors.

Absorption of carbohydrates through the cuticle

The data are summarized in Table III. There is no doubt that ligated flukes

TABLE III.—*Absorption of carbohydrates through the cuticle of* Fasciola hepatica in vitro

	Number of Flukes Surviving In Days							
Group	0	1	2	3	4	5	6	7
Unligated Controls								
No glucose	9	9	8	0				
Glucose	7	7	7	7	7	6	6	6
Ligated								
No glucose	10	10	10	3	2	0		
Glucose	13	13	13	13	13	13	13	12
Glycerol	7	7	7	7	7	7	6	5

were unable to ingest food through their oral openings. By the third day all movement anterior to the ligature had ceased. In the majority of the flukes, the area anterior to the ligature had blanched by this time.

It was not possible to carry these experiments beyond a week's time. By the sixth day the flukes began to blanch behind the ligature. On the seventh day the anterior half to the ligated fluke was very pale, and the animals were barely alive. As this condition proceeded both anteriorly and posteriorly from the ligature, it is attributed to necrosis produced by mechanical pressure of the silk thread.

Utilization of simple energy sources

The data are summarized in Table IV. Glucose, fructose and glycerol were the

TABLE IV.—*Aerobic utilization of simple compounds by* Fasciola hepatica in vitro

	Number of Flukes Surviving						
	In Days						
Compound	0	1	2	3	4	5	6
No organic compound	13	13	9	0			
Glucose	16	16	16	16	14	14	13
Fructose	5	5	5	5	4	4	4
Galactose	9	9	9	2	0		
Ribose	9	7	5	0			
Maltose	9	8	4	0			
Lactose	10	10	7	0			
Sucrose	10	9	8	1	0		
Sorbitol	8	7	5	2	0		
Glycerol	12	12	12	10	10	10	10
Cholesterol	9	9	8	5	0		
Pyruvate	9	9	9	9	6	2	0
Glutamic acid	14	14	12	9	2	0	

only compounds found that would support the parasite for the 6-day period of the experiment. As such clear-cut results were obtained within this period, and as so many other nutrients are essential to prolong survival of the fluke *in vitro*, this short period was considered sufficient. The basic saline used was Tyrode's solution.

Anaerobiosis

The data are summarized in Table V. As there was a possibility that *F. hepatica* was a microaerophilic organism and was obtaining sufficient oxygen during the change of solution, Bacto-Fluid Thioglycollate (DIFCO) was employed to rule out this possibility. It was found that the trematode would not survive 10–12 hours in the recommended strength for bacterial cultures: 29.5 gm medium per liter of distilled water. When this solution was diluted in half with Tyrode's solution, the parasite survived (Table V). In the case of the thioglycollate solution there is no doubt that there was no oxygen available for the fluke; even during the changing of solution, there was no change in the color indicator in the solution surrounding the fluke to indicate the presence of oxygen.

To determine whether or not the parasite utilizes oxygen when it is present, 1 gm sodium pyruvate was substituted for glucose in Tyrode's solution. As the data in Table V show, the parasites in anaerobic solutions all died within 3 days, but all flukes in aerobic cultures were still alive on the third day, and pyruvate alone was able to sustain 2 flukes through the fifth day.

DISCUSSION

The pH of the solution is probably not as critical as Stephenson's (1947) data indicate. The effect noted by Stephenson was probably due more to the control of

TABLE V.—*Comparative survival of* Fasciola hepatica in vitro *under aerobic and anaerobic conditions*

		Number of Flukes Surviving																				
		In Days																				
Solution	Gas Phase	0	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	20	21
Tyrode's	Air + CO_2	8	8	8	8	8	8	8	8x	7	7	1	1	1	1	0							
	N_2	9	9	9	9	9	9	9	9	8	6x	4	4	1	1	1	1	1	1	1	1	1	0
Pyruvate	Air + CO_2	9	9	9	9	6	2	0															
	N_2	8	8	5	0																		
Tyrode's + ALE	Air + CO_2	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	1	0			
	N_2	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	1	0
AB + ALE	Air + CO_2	5	5	5	5	5	5	5	5	5x	4	4	4	3	3	3	3	3x	2x	1	1	1	0
	N_2	5	5	5	5	5	5	5	5	5	5	5	5	5	4	4	4	4	4x	2	1	1	0
Tyrode's + ICL	Air + CO_2	6	6	6	6	6	6	6	6	5	5x	1	1	0									
	N_2	7	7	7	7	7	7	7	5	5	4x	2	2	0									
Tyrode's + Thioglycollate	N_2	5	5	5	5	5	5	5	5	5	4	4	4	4	4	3	0						

x = One fluke removed from experiment as contaminated. Pyruvate = Tyrode's solution with 1 gm. Na pyruvate substituted for the glucose. ALE = Autoclaved Liver extract. ICL = Injection Crude Liver-MRT.

contaminants in his solutions than to a direct effect upon the parasite. Experiments designed to establish the effect of pH were not undertaken when it was found that changing the pH of 8.5 to 7 had little or no effect upon survival. As the fluke *in vivo* is exposed, in its various hosts, to a wide range of pH, from slightly acid to about pH 9, the parasite *in vitro* should also be able to tolerate this range.

An examination of Table I and a comparison with Dawes's (1954) use of Hedon-Fleig solution indicates that *F. hepatica* can tolerate a wide range of inorganic ion concentrations *in vitro*.

It is interesting to note that *F. hepatica* is a rather hardy animal. There were several anterior-halves of flukes (not included in any tables) which survived over a week in AB and Tyrode's solution. One survived in AB plus the 2 liver extracts for three weeks although it was almost cut in two. The appearance and motility of these flukes were not dissimilar to whole flukes during this period. Flukes with the cephalic cone removed died within a week's time.

The action of the cephalic cone appears to be the primary factor in the excretion of waste materials. It was noted that if the cone was cut off at the base, although body constrictions and extensions continued for several days, waste materials remained in the gut and served as foci for necrosis. It was observed that *F. hepatica* disgorges materials by working them medianly out of the lateral pouches of the intestinal cecum, and then anteriorly and out the oral orifice by contractions and extensions of the body and the cephalic cone. The complete expulsion of visible materials may take from 2 to 3 days for a fully engorged fluke *in vitro*, but it is usually accomplished within 24 hours.

It is interesting to note that a few ligated flukes without an energy source lived longer than unligated flukes with no energy source (Table II). Unligated flukes without an energy source were never found to live beyond 2 days in the course of this study. This would indicate that the endogenous reserve material in a fluke is sufficient for a survival of 2 days. In addition, it implies to this author that although foodstuffs may not be visible in the gut, the fluke probably excretes materials through the oral opening which it could metabolize further rather than that the inorganic ions were toxic to the unligated fluke.

The survival in glucose and fructose solutions is in accord with the findings of Stephenson (1947); but contrary to his data, *F. hepatica* will not survive in other sugars. This would imply that *F. hepatica* can metabolize those 2 sugars, but is unable to use the other simple sugars or breakdown the complex ones to products the parasite can use. Undoubtedly during Stephenson's experiments, contaminating microorganisms broke down the complex sugars for the fluke.

Although 2 flukes in the galactose solution, 1 fluke in the sucrose, and 2 flukes in sorbitol lived for 3 days, this investigator does not consider these compounds usable by the fluke. Parasites in the above solutions were very blanched, distorted and barely alive on the third day. On the other hand, cholesterol, pyruvate and glutamic acid, while not supporting the fluke for the 6-day period, seemed to have some beneficial effect, for in these cases over 50% of the parasites were still alive on the third day and were normal in appearance.

The ability of this parasite to survive in pyruvate solution only in the presence of oxygen, the ability to survive for a short time in only glutamic acid plus the ability to utilize this substance as shown by van Grembergen (1949) and Daugherty

(1952), and the demonstration by van Grembergen (1949) of an active succinic dehydrogenase indicate that *F. hepatica* most probably utilizes a mechanism similar to the "Kreb's cycle" for the oxidative metabolism of carbohydrates. When this information is added to the data on respiratory enzymes found in *F. hepatica* by earlier workers (Gourevitch, 1937; van Grembergen, 1949; and Stephenson, 1947), the evidence indicates that this fluke is capable of an aerobic existence. The question remains, however, as to whether or not conditions are such *in vivo* to permit an aerobic existence.

Sobotka (1937) listed the measured values of oxygen in bile between 0.19 and 0.89 volumes per cent. In addition the present investigator noticed that flukes *in vivo* usually lie in close association with the mucosa of the bile duct, particularly in areas where the large blood vessels cross the ducts. Here the oxygen tension should be higher than in the middle of the bile stream, for some of the constituents of bile are readily oxidized. Also during several hundred examinations of infected bovine livers, parasites were never found in the gall bladder which has an extremely low oxygen tension.

However, in addition to the amount of oxygen present, one must consider the size and shape of an animal which has no respiratory apparatus to determine whether or not it is possible for that animal to obtain sufficient oxygen. *Fasciola hepatica* is a thin, flat animal which presents a very large surface area in relation to its volume. On measuring 4 medium-size flukes, it was found that there is approximately 300 mm^2 surface area to a volume of approximately 100 mm^3. These figures represent contracted flukes. When alive and motile, the surface area can be more than double the above figure. It can be seen, therefore, that *F. hepatica* has a large surface area through which oxygen can diffuse.

Prosser (1950) points out that organisms with tissues not more than 0.5 mm distant from the oxygen source can obtain sufficient oxygen by diffusion alone and that "with an adequate oxygen source rapid utilization establishes a high gradient, and diffusion over short distances can be very rapid." From this it would appear that diffusion alone could supply *F. hepatica* with its necessary oxygen.

In addition it can be postulated that the great ramification of the intestinal cecum of this parasite could serve, to a certain extent, as a respiratory apparatus, for the material in the gut ebbs and flows with the movement of the animal. The gut could be considered to be somewhat analogous to the circulatory system in insects which is not the primary transport system of oxygen but does serve secondarily as a carrier of oxygen to tissues not well supplied with tracheoles. The action of the gut could, therefore, serve to distribute dissolved oxygen more evenly throughout the interior of the animal.

Experimentally, flukes in anaerobic Tyrode's solution lost the color of their hemoglobin more rapidly than did those in aerobic solution. Although when Injection Crude Liver was added, the presence or absence of oxygen had little effect upon the color of the parasite. Parasites in anaerobic solutions tended to become less active after about 3 days. As their activity lessened, the flukes tended to contract and become more thickened. This condition is not typical of what is seen *in vivo*. The term "facultative anaerobe" appears to be the most satisfactory with which to describe *F. hepatica*, for it was demonstrated that *in vitro* the fluke can live without oxygen present. Although it appears that normally there is enough oxygen

present in bile to support the parasite, in chronic infections where the bile ducts have become calcified and the bile is mucoid, there is probably little or no oxygen available for the parasite.

SUMMARY

1. Data are presented showing the aerobic maintenance *in vitro* of bacteria-free adult *F. hepatica*. Autoclaved liver extract at the rate of 200 ml to 800 ml of basic saline solution plus 10 ml Injection Crude Liver maintained some flukes in excellent condition for 3 weeks and permitted survival for 30 days.

2. Variation in pH within the range of 7 to 8.5 and considerable variation of inorganic constituents of the basic saline solution did not affect the survival of the parasite *in vitro*.

3. Data are presented which confirm Stephenson's (1947) demonstration that *F. hepatica* can apparently absorb simple carbohydrates through its cuticle.

4. *F. hepatica* can survive in solutions of glucose, fructose and glycerol, and to a lesser extent in cholesterol, pyruvate and glutamic acid. The fluke cannot survive in solutions containing galactose, sucrose, maltose, ribose or sorbitol.

5. Data are presented which show that *F. hepatica* can survive as long in anaerobic solutions as in aerobic solutions, but that the motility, color and shape indicate that this parasite is primarily an aerobic organism. Arguments are presented to support the hypothesis that *in vivo, F. hepatica* lives primarily as an aerobe.

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NITROGENOUS EXCRETORY PRODUCTS OF *TRICHINELLA SPIRALIS* LARVAE

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The literature contains little information on the catabolic processes of nitrogen metabolism of nematode larvae. Studies on the adults of various species indicate that the principal product is ammonia, which amounts to 30 to 50% of the soluble excreted nitrogen in most cases (Weinland, 1904; Rogers, 1952; Savel, 1954). The remainder of the soluble nitrogen is accounted for as polypeptides, proteins, amino acids, small amounts of amines (Weinland, 1904; Flury, 1912; Rogers, 1952, 1955), and also as urea and/or uric acid in some species (Chitwood, 1938; Rogers, 1952; Savel, 1954).

The present report concerns a quantitative evaluation of the soluble nitrogenous materials excreted by *Trichinella spiralis* larvae incubated aerobically in saline under axenic conditions, supplementing previous observations on the carbohydrate and lipid metabolism of this organism (von Brand, Weinstein, Mehlman, and Weinbach, 1952). The advantages of working with bacteria-free larvae were twofold. It was possible to obtain sterile incubates without the use of chemical sterilizing agents or the addition of antibiotics. The latter was important because it obviated the difficulties of separating the nitrogen-containing antibiotics from the excretory products. There was also no possibility of contamination by bacterial metabolites.

MATERIALS AND METHODS

T. spiralis incubates. The methods used for the preparation of the incubates were essentially the same as those described previously (von Brand, Weinstein, Mehlman, and Weinbach, 1952), except for a few modifications. The meat digestion in the present study was performed in a sterile, covered jar containing a Teflon-covered magnetic bar rotated by a magnetic stirrer, the speed of which could be adjusted as digestion proceeded. This was much less cumbersome than the use of the paddle in the former study, and was not as subject to bacterial contamination. All larval suspensions were incubated aerobically at 37°C in 10 ml of 0.7% sodium chloride in 125 ml Erlenmeyer flasks plugged with cotton. Saline was used as the incubation fluid instead of a balanced salt solution, since it was found that salt mixtures interfered with the determination of some substances, e.g. the amines. During the incubation period, the amount of water lost by evaporation gradually increased the concentration of the sodium chloride to approximately 0.8%. The number of larvae incubated per flask varied between 118,000 and 510,000. The flasks were rocked at 25 oscillations per minute for approximately 23 to 24 hours. The incubate, after removal of the larvae by centrifugation, was stable for several days when stored at 4°C. The analyses for the various nitrogenous materials were carried out on suitable aliquots.

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Total nitrogen. Kjeldahl digestion of duplicate 0.2-ml samples was followed by Nesslerization and spectrophotometric determination of the intensity of the color developed. The method used was essentially that described by Johnson (1941).

Ammonia nitrogen. Duplicate 0.2-ml samples were Nesslerized directly for the determination of the nitrogen present as ammonium salts. It was shown that, under the experimental conditions, primary aliphatic amines did not interfere with this determination. This was accomplished by Nesslerizing standard solutions of the hydrochlorides of methyl amine, *n*-butyl amine, ethylene diamine, cadaverine, and ethanol amine containing approximately the same amount of nitrogen as would be encountered in the incubate samples as ammonia nitrogen. In no case was a significant amount of color produced. Cadaverine may develop a fine opalescent precipitate at this concentration (20 micrograms of nitrogen per 0.2 ml) which interferes by screening the light path. This does not occur at the concentrations of cadaverine found in the incubates.

Non-volatile nitrogen. The volatile nitrogen compounds, ammonia and the lower aliphatic primary amines, were removed from duplicate 0.2-ml samples by the addition of 0.1 ml of 2 *N* sodium hydroxide and evaporation to dryness *in vacuo*. The samples were held at 2 to 3 mm pressure in the presence of sulfuric acid for 4 days to insure the removal of all volatile basic nitrogen compounds. The residue was dissolved in 0.2 ml of water; 0.1 ml of 6 *N* sulfuric acid was added and nitrogen determined by the Kjeldahl method. Control experiments were made using the solutions of the aliphatic amines mentioned in the previous paragraph in order to demonstrate that the amines were removed by this procedure. It was found that all were removed with the exception of ethanol amine. Only 80% of this amine could be evaporated under these conditions. Increasing the length of time under vacuum an extra day did not volatilize appreciably more of the compound. Thus the non-volatile nitrogen values reported are slightly high due to this effect. However, the error is small since the amount of amines present is low and the hydroxy amines represent only a fraction of this.

Amino acid nitrogen. Duplicate 0.2-ml samples were treated with 0.1 ml of 0.1 *N* sodium hydroxide and concentrated *in vacuo* to dryness to remove ammonia and volatile amines. The residue was dissolved in 0.2 ml of water and 0.1 ml of 0.1 *N* hydrochloric acid was added. The amino acids were then determined by the ninhydrin method described by Snell and Snell (1954). The blank was 0.2 ml of 0.85% saline carried through the same evaporation procedure. The amino acid standard consisted of 0.2 ml of a 0.0005 *M* solution of a mixture of 19 amino acids commonly found in protein hydrolysates. This solution contained 100 micrograms of nitrogen per ml by Kjeldahl analysis. The standard samples were carried through the alkalization and evaporation procedure in parallel with the incubate samples and the blank. This was later shown to be unnecessary because direct determination of the amino acids in the standard samples gave the same results as those from the samples carried through the evaporation procedure. In order to determine whether protein present in the incubate might interfere either directly or by partial hydrolysis during the alkaline concentration, samples of egg albumin solution in 0.85% saline containing 20 micrograms of nitrogen per 0.2 ml were carried through the entire procedure. The color developed by the ninhydrin was negligible, and it was concluded that the protein did not interfere.

Peptide nitrogen. No entirely satisfactory method for the determination of protein nitrogen in the incubate was found. The amount of precipitate produced by trichloroacetic acid varied with the concentration of the acid, the time, and the temperature. Phosphotungstic acid (2.2%) precipitated considerably more material than trichloroacetic acid did, while 10% tungstic acid produced no precipitate. The general impression obtained was that of a solution containing a variety of proteins and polypeptides with widely distributed molecular weights. The protein determination based on the reaction with Folin's phenol reagent after hydrolysis gave extremely high results amounting to more than the total nitrogen present. This indicated that many more phenolic groups, probably from tyrosine, were present than would ordinarily be encountered in protein hydrolysates. The latter observation was substantiated by the determination of the differential absorption of the incubates in the ultraviolet at 280 and 260 millimicrons (Warburg and Christian, 1942). The optical density ratio 280/260 was less than one, indicating that abnormal amounts of material absorbing strongly at 260 millimicrons were present. Tyrosine and phenylalanine absorb strongly in this region as well as a number of purine bases and their associated nucleotides.

The only determination in which it was felt that some confidence could be placed was the biuret method. This measured the peptide linkages present and thus would include any polypeptides along with the protein. This method required 1-ml samples of the incubate due to its relative insensitivity but gave consistent results even at low color density readings. The procedure was to treat 1-ml aliquots of the incubate with 2 ml of biuret reagent prepared according to Gornall, Bardawill, and David (1949). The mixture was allowed to stand 30 minutes and the optical density determined at 540 millimicrons using 1 ml of 0.85% sodium chloride plus 2 ml of the reagent as a blank. A solution of egg albumin in normal saline was used as a protein standard. This solution contained 15.8 micrograms of protein nitrogen per ml by Kjeldahl analysis. Duplicate 1-ml samples of the standard were run parallel to the incubate samples for each set of analyses.

Volatile aliphatic amine nitrogen. No reliable method for the direct determination of small amounts of aliphatic amine nitrogen in the presence of large amounts of ammonia could be found. An estimation of the amount of volatile-amine nitrogen was obtained by subtracting the ammonia nitrogen from the total volatile nitrogen value.

Urea. Quantitative analyses for urea in the incubates were made by the urease method U-11a.1 described by Fister (1950). In no case was any significant amount of urea found. Control experiments were performed in which known amounts of urea were added to the incubate samples before the determination was made. The results were in agreement with the amounts added, showing that the method was satisfactory and that no inhibitors for the urease were present.

Uric acid. This determination was based on measuring the rate of disappearance of uric acid spectrophotometrically as it was converted to allantoin by uricase by the method described by Mahler, Hübscher, and Baum (1955). No changes in optical density were observed with the incubate samples, but upon addition of a uric acid solution, immediate response was obtained.

Creatine. One-ml samples were examined by the method C-41a described by Fister (1950). No creatine was found.

Preformed creatinine. One-ml samples were examined by the method C-43a described by Fister (1950). No preformed creatinine was found.

RESULTS

The yield of nitrogenous products was calculated as micrograms of nitrogen per 100,000 larvae per 24 hours in the incubate. The yield of nitrogen varied from 400 to 600 micrograms per 100,000 larvae for the 9 incubates examined. In general, the best yields were obtained from incubates which contained approximately 250,000 larvae per 10 ml of saline. Larger numbers caused a reduction in the yield, presumably due to overcrowding.

Table I gives the results in terms of the percentage of the total nitrogen. The first column gives the average of the values from 9 incubates and the second gives the minimum and maximum figures found. It will be noted that an average of 10% of the nitrogen is unaccounted for. This nitrogen is in the non-volatile fraction and consists in part of any amines which were incompletely removed by the vacuum concentration procedure. However, this could not amount to more than 5 to 10% of the total volatile amines present, which in turn would be not more than 1% of the total nitrogen. Thus, there apparently remains some undetermined nitrogen compounds in the residue. It was in search of these that quantitative determinations for urea, uric acid, creatine, and creatinine were made but none could be found. Whether this remaining nitrogen is present as one or more undetermined compounds or merely represents the sum of the errors from the other determinations remains unknown.

TABLE I.—*Type and amount of nitrogen excreted by* T. spiralis *larvae*

	Average (%)	Spread (%)
Ammonia	33.3	28–37
Volatile amine	7.4	2–12
Peptide	20.8	15–28
Amino acid	28.5	25–32
Non-volatile	61.5	59–72
Total nitrogen accounted for	90.0	85–93

DISCUSSION

The nitrogenous compounds which were identified in the incubates were derived from organisms maintained aerobically in a non-nutritive medium and manifesting essentially catabolic activity. Whether the nitrogen partition obtained will change qualitatively and quantitatively when larvae are studied under conditions of optimal nutrition must await the development of adequate *in vitro* culture methods. The analyses, however, are based on axenic incubates and, as such, are not complicated by the by-products of bacterial metabolism which have made previous studies difficult to interpret.

Compared to other nematodes, the total amount of nitrogen excreted by *T. spiralis* larvae was large. Assuming that the dry weight of these larvae is approximately 20% of the wet weight, then, based on the figures of Stannard, McCoy, and Latchford (1938), the wet weight of 100,000 larvae would be roughly 35 mg. This would give an average of 2.8 mg nitrogen per gram wet weight produced in

24 hours. The highest nitrogen yield encountered by Rogers (1952) was for *Nematodirus spp.*, ranging from 1.23 to 1.57 mg nitrogen per gram wet weight in 24 hours.

No attempt can be made at this time to differentiate between nitrogenous products which have arisen by way of the intermediary metabolism and those which originated from glandular secretions, by diffusion through the cuticle, or were eliminated from the gut in partial or complete stages of digestion. The latter source may account for much of the protein-peptide-amino acid complex. Many other invertebrates, however, have been found to excrete as high as 30 percent of their nitrogenous waste in the form of amino acids (Prosser *et al*, 1950, table 30), but as Baldwin (1948) points out, it is not known whether this is simply due to leakage of body fluids or some sort of metabolic disability. Rogers (1952, 1955) has demonstrated the presence of peptides and amino acids in incubates of 2 species of *Nematodirus, Ascaridia galli,* and *Ascaris lumbricoides,* and believes that the evidence suggests that these are true excretory products. The probability is that ammonia and volatile amines constitute the primary nitrogenous products of *cellular* catabolism in *T. spiralis,* and that it may be considered an ammonotelic organism. Other papers (Haskins and Weinstein, 1956a, b) characterize the species of amines and amino acids which were found in the incubates.

SUMMARY

Trichinella spiralis larvae were incubated aerobically under axenic conditions. Chemical analysis of the incubates showed the presence of 400 to 600 micrograms of nitrogen per 100,000 larvae per 24 hours incubation. The nitrogenous products consisted of 33.3% ammonia nitrogen, 7.4% volatile amine nitrogen, 20.8% peptide nitrogen, and 28.5% amino acid nitrogen with 10% of the total nitrogen unaccounted for.

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RESEARCH NOTE

THE REACTION OF *SCHISTOSOMA MANSONI* EGG SHELLS TO THE PERIODIC ACID-SCHIFF STAINING PROCEDURE

Lillie (1947, J. Lab. & Clin. Med. **32:** 76–88) reported that the egg shells of *S. mansoni* and *S. japonicum* in tissues are Schiff-positive, staining usually deep purplish-red, when treated by the Bauer technique for polysaccharides. More recently, in a personal communication to one of us (G. M. C.), Lillie wrote that positive results have also been obtained for *S. mansoni* eggs when stained by the periodic acid-Schiff (PAS) method. The same results have been obtained with this technique for *S. japonicum* eggs in liver tissue (Sawada *et al*, 1956, Am. J. Trop. Med. and Hyg. **5:** 847–859). Because this is contrary to what we have observed in a study of *S. mansoni* eggs in tissue, it is of interest to discuss this particular matter.

The tissues used in our experiments were obtained from mice (infected for not more than 57 days), fixed for 24 hours in Bouin's picro-formol-acetic acid fluid, washed in several changes of 70% ethyl alcohol for at least 24 hours, dehydrated, cleared in xylol, and embedded in Fisher Tissuemat. The McManus (1948, Stain Technol. **23:** 99–108) periodic acid leuko-fushsin (Schiff stain) technique was used, but instead of the sulfurous acid rinses after the Schiff's reagent, three baths of M/20 (0.52%) sodium bisulfite (C.P. grade), as suggested by Lillie in his text (Histopathologic Technic, 1948, Blakiston Co., Philadelphia), were employed. Slides of alternate serial sections were prepared so that tissues carried through the PAS procedure would be accompanied by control slides treated exactly as the test slides except that they were not subjected to treatment with periodic acid.

The egg shells were definitely PAS-negative. However, at times a strongly PAS-positive substance was found adhering to the borders of the shell causing the shell to appear to be positive. Fragments of shell in absence of larvae were also negative. This, too, is contrary to the findings of Lillie (1947) who reported that fragments of schistosome eggs stained by the Bauer method are also positive.

Lewert and Lee (1954, J. Inf. Dis., **95:** 13–51) used the Hotchkiss-PAS method and, in discussing the positive staining material in the glands of miracidia of eggs passing through host tissue, state the following (p. 23): "In normal eggs this material is not only present within the egg shells but appears to pass through it, forming an exterior coating about the shell. . . . This is particularly well shown in frozen-dried material and is usually not apparent or is less apparent in materials fixed by more usual methods." Although illustrations in this paper indicate that the shells remain unstained, this fact is not emphasized in the text. In a personal communication to one of us (F. F. K.), Lewert clarified this point and confirmed our observations that the egg shells of *S. mansoni* are PAS-negative. In addition, he pointed out that this is also true for *S. hematobium* and *Schistosomatium douthitti*.—FRANK F. KATZ AND G. M. CARRERA, *Department of Tropical Medicine and Public Health, Tulane University, New Orleans, Louisiana. Present addresses: Eaton Laboratories, Norwich, New York, and Ochsner Foundation Hospital, New Orleans, Louisiana, respectively.*

AMINO ACIDS EXCRETED BY *TRICHINELLA SPIRALIS* LARVAE

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An analysis of the nitrogenous excretory products obtained from *Trichinella spiralis* larvae incubated aerobically in saline under axenic conditions has been reported previously, and it was found that 25 to 30% of the nitrogen fraction was present as free amino acids (Haskins and Weinstein, 1956 a, b). Rogers (1955) has observed the excretion of amino acids during aerobic incubation of adult *Ascaris lumbricoides, Ascaridia galli,* and *Nematodirus* spp., and has shown that there was little change in the character of the amino acids under anaerobic conditions. The present report concerns the specific identification of the amino acids present in the incubates of *T. spiralis* larvae.

PROCEDURE AND RESULTS

The preparation of the saline incubates of the *T. spiralis* larvae was made in the same way as described for the determination of the nitrogenous excretory products (Haskins and Weinstein 1956a). The larvae-free incubates obtained from 150,000 to 200,000 organisms were used to prepare the samples for identification of the amino acid constituents by paper chromatography. The general procedure was the evaporation to dryness *in vacuo* of a suitable aliquot of the incubate, usually 1 ml. The dry residue was dissolved in 0.1 ml of 0.1 *N* hydrochloric acid which was neutralized with an equivalent volume of 0.1 *N* sodium hydroxide immediately before application to the paper. The solutions were not desalted because of the small amounts of material involved and because control experiments with mixtures of known amino acids under the same conditions gave satisfactory chromatograms in which the individual amino acids were readily identified. Samples from the concentrate were applied to the center of 24-cm circular Whatman No. 1 filter paper 5 microliters at a time until 80 microliters had been applied. The chromatograms were processed at room temperature (25°C) by the technique described by Oreskes and Saifer (1955). The solvents were *n*-butanol (40)-acetic acid(10)-water(50) and phenol(70)-isopropanol(5)-water(25). The phenol runs were made in the presence of 0.1% ammonia solution. The butanol solvent was removed by a current of air, the phenol by washing twice with acetone and air-drying. Thereafter, the papers were divided in half along a diameter, and one half dipped in a 0.25% solution of ninhydrin in acetone. The other half was dipped in a 0.2% solution of isatin in acetone containing 4% acetic acid. The colors were developed by heating at 100°C for 5 minutes. Control chromatograms with mixtures of known amino acids were run under the same conditions as a check on the Rf values and as an aid in identifying the amino acid constituents. The Rf values agreed very well with those reported by Oreskes and Saifer (1955).

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It was conceivable that the amine constituents of the incubates might interfere in the identification of the amino acids (Haskins and Weinstein, 1956 b). In order to test whether the amines present were in sufficient quantity to produce interference or to be confused with the amino acids, duplicate 1-ml samples of an incubate were treated, one with 0.1 ml of 0.1 *N* hydrochloric acid and the other with 0.2 ml of 0.1 *N* sodium hydroxide. Both were concentrated under reduced pressure to dryness and held at 2 to 3 mm pressure for 4 days in the presence of sulfuric acid and solid sodium hydroxide. This insured the removal of the volatile amines, with the possible exception of a fraction of the hydroxyamines (Haskins and Weinstein, 1956 a) from the alkaline sample, and retention of all amines present in the acid sample. The dry residues were dissolved in 0.1 ml of water and the pH adjusted to 4 with 0.1 *N* acid or alkali, as required, before application to the paper. The chromatograms produced by both samples were essentially identical. It was concluded that, under these conditions, any amines present in the samples were in such low concentrations as to produce no interference with the identification of the amino acids. Control chromatograms were also prepared from concentrates of the last saline wash of the larvae before incubation. In no case were there any contaminating substances carried over into the incubates as evidenced by the absence of any bands appearing on these chromatograms.

In all, 7 samples of incubate concentrates were examined and the following amino acids were identified: glutamic acid, serine, glycine, alanine, tyrosine, valine, methionine, leucine, phenylalanine, and proline. The method does not distinguish between leucine and isoleucine, so one or both may be present. In 1 experiment the paper from each solvent was cut into 8 pie-shaped pieces. One piece from each was developed with ninhydrin to locate the bands. The other pieces were developed with a number of special reagents for confirmatory tests for certain amino acids. Table I gives the results of these tests.

DISCUSSION

With few exceptions, the free amino acids found among the excretory products of *T. spiralis* larvae were the same as those identified by Rogers (1955) from adult nematodes; serine, glycine, and methionine were found present and aspartic acid absent in the case of *T. spiralis* larvae.

TABLE I.—*Confirmatory tests on amino acid chromatograms*

Substance	Result	Reagent
Cystine	absent	Iodine-sodium azide
Cystine	absent	Platinic iodide
Cystine	absent	Nitroprusside and cyanide
Tryptophane	absent	*p*-Dimethylaminobenzaldehyde
Tyrosine	present	Diazotized sulfanilic acid
Histidine	absent	Diazotized sulfanilic acid
Histidine	absent	Diazotized *p*-nitroaniline
Arginine	absent	Sakaguchi

Two bands having relatively low Rf values with both butanol (0.15 and 0.22) and phenol (0.12 and 0.21) solvents were not identified. Initially, the inner band was tentatively designated as cystine, but, as shown in table I, no confirmatory tests for this substance could be obtained. It is possible that these bands are similar to the "peptide" bands described by Rogers (1955).

It is interesting to note that, although tyrosine and phenylalanine are present, the corresponding tyramine and phenylethyl amine were not found among the amine constituents (Haskins and Weinsteins 1956 b). Otherwise, each of the amino acids, with the possible exception of proline and glutamic acid, have an amine present corresponding to the simple decarboxylation of the acid. Pyrrole and 4-aminobutanoic acid were not observed as amine constituents but, since no special tests were made to determine their presence, it is possible that they might be present in small amounts.

SUMMARY

Trichinella spiralis larvae were incubated aerobically in saline under axenic conditions. The saline incubates were concentrated and examined for amino acids by paper chromatographic techniques. The following amino acids were identified: glutamic acid, serine, glycine, alanine, tyrosine, valine, methionine, leucine, phenylalanine, and proline. Amines present in the incubates were shown not to interfere with the identification of the amino acids under the experimental conditions used.

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RESEARCH NOTE

AN UNUSUAL *TRIATOMA* FEEDING RECORD

While collecting *Triatoma* on the Welder Wildlife Refuge near Sinton, Texas, June 12, 1956, an observation was made of sufficient interest to report. Adjacent to the temporary Refuge headquarters a small box of firewood was emptied in a search for *Triatoma* bugs. At the bottom, a single fifth instar *T. gerstaeckeri* nymph was discovered in close association with an adult tropical indigo snake, *Drymarchon corais*. The bug had recently engorged and as there was no other apparent source of blood it was considered likely that the snake was the host animal.

A blood film was made of the bug's stomach contents and the erythrocytes were found to be nucleated. The blood was also teeming with microfilaria which Dr. A. C. Chandler, Rice Institute, has indicated resemble *Cardianema cistudinis*, described by Alicata in 1933 from the North American box turtle.

Triatoma readily feed on a wide variety of non-mammalian hosts in the laboratory, especially reptiles (Wood, 1944, J. Parasitol. **30:** 197; Ryckman, 1954, Trans. Am. Micr. Soc. **73:** 215). Abalos and Wygodzinsky (1951, An. Inst. Med. Res., Tucuman, Argentina, Mon. 2, 178 pp.) fed *Triatoma* on a spider and silkworm larvae. Packchanian (1940 Pub. Health Repts. **55:** 1527) tells of *T. sanguisuga* feeding on tree toads of the genus *Hyla* in Florida palm trees. However, reports of these bugs feeding on animals other than mammals in nature are not common.—R. B. EADS, *Texas State Department of Health, Austin.*

THE AMINE CONSTITUENTS FROM THE EXCRETORY PRODUCTS OF *ASCARIS LUMBRICOIDES* AND *TRICHINELLA SPIRALIS* LARVAE

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It has been reported in previous publications (Weinstein and Haskins, 1955; Haskins and Weinstein, 1956a) that among other nitrogenous wastes, amines are excreted by the larvae of *Nippostrongylus muris* and *Trichinella spiralis*. Those of *N. muris* have been previously identified as a miscellany of primary aliphatic amines. The present work is an extension of these studies, determining the identity of the amines excreted by *T. spiralis* and *Ascaris lumbricoides* larvae under axenic conditions.

MATERIALS AND METHODS

In general, the biological and chemical techniques applied were the same as those described in the aforementioned papers.

T. spiralis. Incubates were derived from flasks containing from 227,000 to 562,000 larvae, using the same methods previously given (Haskins and Weinstein, 1956a).

A. lumbricoides. (a.) *Preparation of incubates.* Eggs were isolated from the anterior portion of the uteri of worms obtained from pigs, and were placed in 1% formalin until embryonation was completed. The age of the embryonated eggs used in these experiments varied from 3 to 7 months. Sterile larvae were obtained using a modification of the procedure of McRae (1935). Embryonated eggs were transferred to approximately 5 ml of Clorox (sodium hypochlorite, 5.25% by weight), and exposed for 4 hours at 37°C. This treatment sterilized, as well as removed all the shell membranes from the eggs (de-shelled eggs), leaving intact the thin, inner, lipid vitelline membrane which served to protect the enclosed larva from injury (Jacobs, 1940, for discussion of membranes). The hypochlorite was removed by washing the de-shelled eggs several times with sterile saline. They were then transferred to a tube containing sterile sand and saline, which was rubber-stoppered. This was vigorously shaken for 60 to 90 seconds, which ruptured the vitelline membranes resulting in approximately 99% of the larvae being hatched. Although a small percentage of the larvae were killed or injured in the process, the great majority were in good condition. The larvae were washed through several tubes of saline by centrifugation. The ruptured vitelline membranes, which formed a distinct layer above the sedimented larvae, were removed and discarded during the washing process. The last saline wash fluid was analyzed for the presence of amines as a control procedure.

The washed larvae were placed in 10 ml of 0.7% sodium chloride solution in a 125-ml flask and incubated in the same manner as the *T. spiralis* larvae for 20 to 24 hours. The larvae were then removed by centrifugation, and the incubate

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fluid was analyzed. Sterility tests were performed by inoculating a few drops of the larval suspension into thioglycollate broth at the beginning and end of the incubation period. Suspensions contained from 600,000 to 3,000,000 larvae per flask.

(*b.*) *Isolation of egg fluid for analysis.* Eggs were treated with hypochlorite to remove the shell membranes, and were then thoroughly washed in saline as described above. The saline suspension was acidified by adding a few drops of 0.05 *N* hydrochloric acid, and the vitelline membranes were ruptured by shaking with sand. The fluid surrounding the larvae within the eggs was thus liberated into the acid-saline, as were the larvae. The ruptured membranes and larvae were removed by centrifugation, and the supernatant fluid containing the egg fluid was analyzed for the presence of amines.

A small number of larvae were usually macerated by the sand in the above procedure, and in order to avoid contaminating the egg fluid with larval-body substance, the vitelline membranes were ruptured in the following manner also. The de-shelled egg suspension was prepared in acid-saline, and to this a few drops of xylene were added. The suspension was incubated at 37°C for 45 minutes, during which time it was shaken at intervals. The vitelline membranes dissolved completely, liberating the egg fluid and the larvae. The xylene was vaporized by bubbling air through the emulsion, and the larvae were then removed by centrifugation. The supernatant fluid was analyzed for amines.

Chemical. The incubates were acidified with hydrochloric acid and evaporated to dryness under reduced pressure. The dry residue was extracted with absolute alcohol and these extracts, upon evaporation to 0.1 to 0.2 ml, were used to spot the Whatman No. 1 paper for the chromatographic analyses. The chromatograms were developed by the descending method using butanol (40), acetic acid (10), and water (50) on one sample, and phenol (80) and water (20) on another. The amines were located by treatment with an 0.1% solution of ninhydrin in chloroform. The identity of the compounds was established by comparison as to location and Rf value with mixtures of known amines chromatographed on the same sheet of paper. Control samples, prepared from alcoholic extracts of the residues obtained from evaporation of the final wash fluids from the larvae before incubation, were also chromatographed by the same technique. In addition, flasks containing only saline were incubated on the rocker apparatus, and these fluids were analyzed chromatographically in the same way. In no case were any contaminating substances detected on these control chromatograms.

RESULTS AND DISCUSSION

A total of 4 incubates from *T. spiralis* and 6 incubates from *A. lumbricoides* larvae were examined. Table I gives the data obtained, compared to those of the known amines which gave spots corresponding to the ones from the extracts.

It was found that *T. spiralis* larvae at the termination of the incubation periods were invariably 99% alive, very active, and in normal morphological condition. *A. lumbricoides* larvae, however, were usually in poor condition at the end of incubation, many being dead. Since is was possible that, in the case of the *Ascaris* larvae, the amines represented byproducts of dying organisms only, an analy-

sis was made of the fluid surrounding the larvae within the egg shells. This fluid should contain the excretory products of the metabolizing larvae under physiologically appropriate conditions within the protective shell. The presence of amines in such fluid should prove their normal production by the organisms. Such fluids were obtained for analysis using both sand and xylene respectively as described in Methods. Approximately 2,000,000 eggs were ruptured using each procedure. With both techniques, the chromatograms were identical to those from the larval incubates with respect to the number and identity of the amines present. The amount of material in the alcoholic extract of the egg fluid appeared to be about equivalent to the amount that would be recovered from the incubation of a similar number of larvae in saline solution.

The identification of allyl amine as one of the amine constituents remains somewhat open to question since no authentic sample was available for chromatographic comparison with the incubate extract. Dihlman (1954) reported that volatile unsaturated aliphatic amines could be distinguished from similar saturated amines by paper chromatography using butanol-acetic acid as solvent followed by development with sodium 1,2-napthoquinone-4-sulfonate in sodium carbonate solution (Folin's reagent); the unsaturated amines gave a yellow-green spot while the saturated compounds gave a blue-gray spot. Since one of the spots on the chromatogram had an Rf value which corresponded to that obtained by Dihlman for allyl amine, and which did not seem to correspond to any saturated aliphatic amine, chromatograms from 2 *T. spiralis* incubate extracts were prepared with butanol-acetic acid and developed with Folin's reagent. In each case the spots having an Rf value of 0.46 had a yellow-green color while all the other spots were blue-gray. The sensitivity of the test is given by Dihlman as 5 micrograms of the amine. These results indicate that allyl may also be present among the various amines in the incubate. A spot with the same Rf value was obtained from similar chromatograms of *A. lumbricoides* larvae incubate extracts; thus allyl amine is also probably present among its excretory

TABLE I.—*Chromatographic identification of amines*

Rf of spot from alcoholic extract from		Corresponding amine	Rf of corresponding known amine
T. spiralis	*A. lumbricoides*		
		Solvent: butanol-acetic acid	
0.07	0.08	Mixture of aliphatic diamines	0.07
0.11	0.12	Unknown	
0.15	0.17	Unknown	
0.22	0.23	Ethanolamine	0.22
0.27	0.27	Methyl amine	0.25
0.32	0.34	1-Amino-2-propanol	0.34
0.39	0.40	Ethyl amine	0.38
0.44	0.45	Allyl amine	0.47**
0.50	0.52	Propyl amine	0.50*
0.58	0.61	Butyl amine	0.59*
0.74	absent	Amyl amine	0.74*
0.82	absent	Heptyl amine	0.82*
		Solvent: phenol-water	
0.18	absent	Ethylenediamine	0.19
0.32	0.29	Cadaverine	0.32
0.37	0.36	Unknown	
0.45	0.43	Unknown	
0.54	0.53	Ethanolamine	0.54
0.70–0.90	0.70–0.90	Mixture of aliphatic monoamines	0.70–0.90

* Rf is for the normal isomer. The isomer actually present in the alcoholic extract was not determined.

** Value reported by Dihlman (1954).

products. No spot coresponding to allyl amine was observed in the case of chromatograms from extracts of *N. muris* larvae (Weinstein and Haskins, 1955).

It is apparent that *T. spiralis* and *A. lumbricoides* larvae produce an even greater variety of aliphatic amines than do *N. muris* larvae. This is particularly true of *T. spiralis* where a total of 11 amines were identified. *Ascaris* larvae were lacking in only 3 of the identified amines; namely, heptyl and amyl amine and ethylene diamine.

It was postulated in the case of *N. muris* larvae that the amines might result from the decarboxylation of various a-amino acids with the single exception of ethylene diamine. This concept can be extended to cover the additional amines found in the present study, but 3 more exceptions must be added to account for the amyl, heptyl and allyl amines. With the growing list of exceptions it appears that mechanisms other than the decarboxylation of a-amino acids which may account for the production of amines are operating in the physiological system.

It should be pointed out that, while the identification of the amines by the chromatographic procedure is reasonably certain, it does not constitute an unequivocal proof of their presence. Later work has shown that, in the case of *T. spiralis* at least (Haskins and Weinstein, 1956b), a considerable amount of amino acids is also present in the incubate. While the solubility in absolute alcohol of the amino acids involved is quite low, in a mixture as complex as that obtained by evaporation of these incubates, the solubility of individual components may be greatly altered. Thus there is some danger of confusing amines with spots produced by amino acids. In appreciation of this possibility, incubates from *T. spiralis* and *A. lumbricoides* were made alkaline and extracted repeatedly with ether. The amines were recovered from the ether by extraction with dilute hydrochloric acid and chromatographed. The chromatograms obtained agreed very well as to both location and Rf value of the spots with those found for the alcoholic extracts. This is another confirmatory piece of evidence that the spots from the incubate extracts are produced by the same materials as the known amines which they resemble chromatographically. However, until sufficient quantities of the individual amines can be isolated for identification by their physical and chemical properties, no absolute claim to their identity can be made.

SUMMARY

Trichinella spiralis and *Ascaris lumbricoides* larvae were incubated in saline under axenic conditions for 24 hours. Alcoholic extracts of the acidified concentrates of these incubates were examined for the presence of aliphatic primary amines by paper chromatographic techniques. *T. spiralis* larvae were shown to produce methyl, ethyl, propyl, butyl, amyl, and heptyl amines, ethylene diamine, cadaverine, and two hydroxy amines, ethanol amine and 1-amino-2-propanol. Allyl amine was also provisionally identified. *A. lumbricoides* larvae produced all of these amines with the exception of amyl and heptyl amines and ethylene diamine.

The egg-fluid inside the vitelline membrane surrounding the larvae of *A. lumbricoides* was also examined by the same technique and was shown to contain qualitatively the same amines present in the incubates from the larvae.

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RESEARCH NOTE

EFFECTS OF *SCHISTOSOMA MANSONI* INFECTIONS ON THE ABILITY OF MICE TO DIGEST AND ABSORB DIETARY FATS AND PROTEINS

In connection with a study on the nutritional aspects of schistosomiasis, it was found that mice infected with *Schistosoma mansoni* did not utilize dietary fats and proteins as efficiently as uninfected control animals. Mice fed a complete semisynthetic dry ration containing casein, sucrose, vegetable oil, salts, and a vitamin mixture plus L-cystine were exposed 4 weeks after they were weaned to 150 *Schistosoma mansoni* cercariae each. A group of comparable mice were held as uninfected controls.

The ability of the mice to digest and absorb fats and proteins was determined over a 3-day period during the eighth week of infection. Estimation of the fat content of the feces was made by Saxon's technique (Saxon, G. J. 1914 J. Biol. Chem. **17:** 99–102) and protein content was determined by standard Kjeldahl procedures. The results of this study are summarized in Tables I and II.

TABLE I.—*Effect of* S. mansoni *infection on dietary fat utilization*

Group	Number of mice	Grams food eaten in 72 hour period[a]	Grams fat eaten in 72 hours	Grams fat recovered from feces	Grams fat utilized	Percent fat utilized
Infected	11	8.9 ± .6	1.77 ± .11	.29 ± .02	1.48 ± .10	83.0 ± 1.6
Uninfected	10	10.0 ± .4	1.99 ± .09	.12 ± .01	1.87 ± .09	93.9 ± 0.6

[a] Contained 19.9 percent fat.

TABLE II.—*Effect of* S. mansoni *infection on dietary protein utilization*

Group	Number of mice	Grams food eaten in 72 hour period[a]	Grams prot. eaten in 72 hour period	Grams prot. recovered from feces	Grams protein utilized	Percent protein utilized
Infected	12	9.3 ± .4	2.69 ± .07	.33 ± .02	2.36 ± .07	87.8 ± .4
Uninfected	9	11.1 ± .3	3.21 ± .10	.23 ± .01	2.98 ± .08	92.9 ± .4

[a] Contained 28.8 percent protein.

It is seen that infection with *S. mansoni* lowered the efficiency of fat utilization from 93.9 ± 0.06% to 83.0 ± 1.6% and for protein utilization from 92.9 ± 0.4% to 87.8 ± 0.4%. These differences are statistically highly significant. *S. mansoni* infections possibly produce these effects by diminishing the digestive functions of the liver and/or by producing acute inflammation of the intestinal tract due to the deposition of large numbers of eggs.—WILLIAM B. DEWITT, *U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Tropical Diseases, Bethesda, Maryland.*

FURTHER OBSERVATIONS ON THE GROWTH OF *TOXOPLASMA GONDII* IN ROLLER TUBE CULTURES OF MOUSE AND PRIMATE TISSUES* **

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The variable symptom-complex produced by *Toxoplasma gondii* in man gradually is being elucidated but little is known of the bionomy of this organism. Studies of its behavior in tissue culture were therefore initiated, and, in an earlier communication, propagation of *T. gondii* in roller cultures of mouse and of human tissue was described (Chernin and Weller, 1954). We now report an extension of these observations and also note the *in vitro* cultivation of this organism in monkey testicular tissues.

MATERIALS AND METHODS

The technical procedures employed and the strains of *Toxoplasma* utilized have been described (Chernin and Weller, 1954), and only pertinent details will be outlined here. Multiplication of *Toxoplasma* in roller tissue cultures was followed in 3 ways: (a) cultures were observed daily using a microscope magnification of 100×; (b) the number of organisms present in the fluid medium was determined by hemacytometer counts; and (c) further quantitative data were obtained by titrations performed *in vitro* and *in vivo*. Counts or titrations were carried out on pooled fluids harvested from a group of cultures, usually 3 to 5 in number. Hemacytometer counts were routinely done in duplicate and the results expressed as an average. For titration, serial ten-fold dilutions of the pooled fluids were prepared in fresh tissue culture medium. Aliquots (0.1 ml) of the respective dilutions were then introduced into each of 3 mouse embryo tissue cultures, and also inoculated intraperitoneally in similar amounts into each of 5 or 6 3-week-old Swiss white mice (Webster Strain). Each titration was controlled by the concurrent inoculation of tissue cultures and mice with 0.1 ml volumes of undiluted nutrient fluid derived from comparable control tissue cultures.

OBSERVATIONS AND COMMENTS

A. *General observations on the behavior of the RH and KM strains of* T. gondii *in mouse embryo cultures.*

1. The RH strain was maintained for 23 passages during a continuous period of 138 days, and the KM strain for 13 passages (93 days) in roller cultures of mouse embryo tissues. At the end of these experiments, the dilution of the original inoculum was 10^{-55} for the RH strain and 10^{-32} for the KM strain. In the course of these passages specific cytopathic changes were observed in all of the 113 roller cultures inoculated with *T. gondii* while in only 4 of 88 control cultures was there evidence of non-specific cytopathology.

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The two strains behaved similarly in culture and a characteristic sequence of events occurred in each passage. After a lag period, free organisms appeared in the culture fluid, increased in number with concurrent evidence of progressive tissue damage, and then disappeared, coincident with apparent complete tissue destruction. This sequence, as observed following the introduction of relatively large inocula, is summarized in Table I. It is to be noted that when minimal infective doses were

TABLE I.—*Sequence of events usually observed by direct microscopy in mouse embryo roller cultures inoculated with* T. gondii

Toxoplasma		Tissue Cells		Tissue Metabolism*
Number observable	Culture Day	Cytopathologic Changes	Culture Day	
None apparent	0–3	None apparent	0–4	Normal
Small to moderate number of individual organisms. Occasionally small groups.	3–6	Slight and focal	4–7	Normal
Large numbers of organisms. Many in sheet-like plaques.	5–8	Generalized, diffuse cellular destruction.	6–9	Slightly reduced
Number of organisms decreased. Plaques smaller and less numerous.	7–10	Cellular destruction marked or complete.	8–10	Markedly depressed
Few or no organisms remain.	10 +	Cellular destruction complete.	10 +	None

* Judged by *p*H differences between infected and control cultures.

introduced, the lag period was extended by as much as 4 or 5 days. However, even with large inocula, variation in the time relationships in this sequential process occasionally occurred without obvious explanation. For example, during the first 13 passages of the RH strain, free organisms consistently appeared 3 to 6 days after inoculation; yet, in the 14th and 15th passages, organisms did not appear until the 7th to 9th day. In subsequent passages, organisms again were noted between the 4th and 7th days of subcultivation.

2. Ordinarily, the nutrient fluids were removed and replaced with fresh medium at intervals of 3 to 4 days, but in one experiment the effect of varying the pattern of replacement of nutrient fluid was investigated. Nine roller cultures of mouse embryo tissue were each inoculated with 0.1 ml of infective fluid from the 16th RH tissue culture passage (inoculum contained 125,000 organisms by hemacytometer count). These cultures were then divided into 3 groups. In Group I no further changes of medium were made. In Group II, the fluid was replaced on the 4th day and then at 3- to 4-day intervals as routinely practiced. The medium in the third group of 3 cultures was first replaced at the 4th day, and then changed daily thereafter. Starting on the 6th day after inoculation, 0.2-ml aliquots of fluid were removed from each tube, pooled and mixed by group, and the number of organisms per ml in each pool determined by hemacytometer count. In the case of the cultures in Group I, the daily loss of medium was corrected every 3rd day by the addition of 0.6 ml of fresh solution. The results of this experiment, summarized in Fig. 1, demonstrate the striking reduction in the yield of organisms from those cultures maintained without change of nutrient (Group I). Differences in the results obtained in the other two groups are less significant since in one of them (Group III) daily changes of medium prevented the accumulation of parasites. It is to be

observed that the numerical counts do not differentiate viable from non-viable organisms.

3. To obtain data on the rapidity of cell invasion and on the mode of intracellular reproduction of *T. gondii,* a series of mouse embryo coverslip cultures were prepared in roller tubes. In one experiment, the medium was removed from 7 4-day-old cultures and replaced with equal volumes of a suspension of *Toxoplasma* obtained from the 7th tissue culture passage of the RH strain; the ID_{50} of this material for mouse tissue cultures was subsequently found to be 10^{-6}. Control cultures received fluid from the comparable uninfected cultures of the 7th passage. The inoculated roller cultures were incubated and coverslips were removed after periods of 3, 6, 20, and 24 hours, fixed and stained. Microscopic examination (970 ×) of 2 prepa-

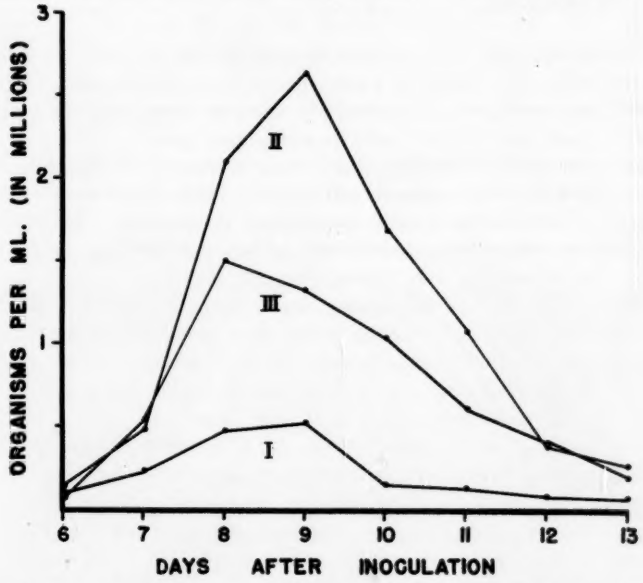

FIGURE 1. The effect of replacement of nutrient medium on the multiplication of *Toxoplasma gondii* in roller cultures of mouse embryonic tissue. Group I cultures: Medium not changed. Group II cultures: Medium changed at 3–4 day intervals. Group III cultures: Medium changed daily after 4 days (See text for details).

rations removed at 3 hours revealed no cells containing identifiable organisms although small basophilic intra-cytoplasmic bodies, resembling those described by Vischer and Suter (1954), were occasionally seen. In the material fixed 6 hours after exposure, a small proportion of cells contained typical parasites; parasites were numerous in the preparations made at 20 and at 24 hours. Counts were made of the number of parasites per infected cell and the results are shown in Table II. At 6 hours, 50% of the infected cells contained 1 organism, 30% contained 2 organisms, and the remainder had from 3 to 10 per cell. The striking feature in the 20- and 24-hour preparations was the frequency with which infected cells contained organisms in multiples of 2 and the paucity of cells containing odd numbers of *Toxoplasma,* thus indicating that reproduction was probably occurring by simple binary

TABLE II.—*Per cent frequency distribution of the number of organisms per infected cell at intervals after the inoculation of cultures with* T. gondii

Number of *Toxoplasma* per Infected Cell	Per cent of Infected Cells Containing Given Number of Organisms at:		
	6 hours*	20 hours	24 hours
1	50	5	15
2	30	21	20
3	6	9	1
4	2	28	20
5	2	2	1
6	4	5	12
7	2	2	0
8	2	18	14
9	1	1	0
10	1	2	1
11 +	0	7	16

* Percentages are based at 6 hours on counts of 50 infected cells. At 20 and 24 hours percentages are based on 100 infected cells.

fission. Similar observations as to mode of reproduction have been reported as the result of the study of *T. gondii* in mammalian macrophages *in vitro* (Vischer and Suter, 1954), and by Pulvertaft *et al* (1954) based on direct observations of the behavior of *T. gondii* in a variety of cells on serum-agar culture.

In other experiments, coverslip cultures were inoculated with materials from the 2nd, 4th, or 7th RH culture passages and fixed thereafter at intervals ranging from 1 to 10 days. Examination of these preparations revealed that both fibroblast-like and epithelial-like cells were susceptible to invasion and were ultimately destroyed. Other than that organisms were characteristically found in cytoplasmic vacuoles, the presence of intracellular *Toxoplasma,* even in large number, appeared to induce little overt cytologic change during the period prior to cell dissolution.

4. Observation of roller tubes of mouse embryonic tissues infected with *T. gondii* suggested that the parasite eventually destroyed all of the tissue cells. To study this further, 3 mouse embryo cultures each were inoculated with 0.1 ml of a 1:10 dilution of a *Toxoplasma*-rich pool from the 7th RH passage; 3 additional cultures were maintained as controls and the medium in all cultures was changed at 3- to 4-day intervals. In the 3 inoculated cultures, organisms became numerous between the 6th and 8th day at which time extensive tissue destruction was apparent. Observation of these cultures for an additional period of 35 days revealed no evidence of tissue metabolism or regeneration, although active tissue growth continued in the control cultures maintained in parallel. It was concluded that under the conditions of the experiment the tissue phase of the cultures containing *T. gondii* had been damaged beyond the point of recovery.

5. To investigate the possibility that the cytopathic changes might be attributable to the presence of a stable soluble toxin, the following experiment was performed. The nutrient fluids were harvested from 4 infected cultures (15th passage RH strain) at a time when free organisms were numerous; these fluids were pooled and centrifuged for 25 minutes at 2000 rpm. The nutrient medium was then removed from 3 fresh tissue cultures, and replaced in equal volume by the organism-free supernatant fluid from the infected tubes. Two control cultures were nourished with pooled fluids from control cultures of the 15th RH passage. Daily examination over a 5-day period revealed no differences in tissue growth or metabolic activity between the experimental and control groups of tissue cultures. While this experiment did not demonstrate the presence of a stable, soluble toxin, it is to be

noted that Vischer and Suter (1954) observed that the rupture of *Toxoplasma*-infected macrophages resulted in damage to adjacent, uninfected cells.

B. *Comparative assay of* in vitro *and* in vivo *infectivity of cultured* Toxoplasma.

At irregular intervals during the serial cultivation of the RH strain, titrations of pooled fluids harvested from infected cultures were simultaneously performed *in vivo* and *in vitro*. The results of infectivity determinations on these pools (Table III) were calculated by the method of Reed and Muench (1938) and expressed as ID_{50}/ml for the cultures and LD_{50}/ml for the mice. In each titration cultures were observed for a minimum of 14 days and mice for a minimum of 17 days. As a further test of possible alteration of pathogenicity, a new isolate of the RH strain

TABLE III.—*Infectivity titers for mouse embryo tissue cultures and mice of* Toxoplasma gondii *suspensions derived from selected passages during serial* in vitro *cultivation in mouse embryonic tissue*

Titration of RH Passage	No. Organisms per ml. of Pooled Inoculum**	ID_{50}/ml. in Roller Cultures	LD_{50}/ml. in Mice
7	Not done	$10^{-6.0}$	$10^{-6.0}$
15	2,680,000	$10^{-5.7}$	$10^{-5.5}$
18	2,175,000	$10^{-4.5}$	$10^{-6.4}$
22	1,850,000	$10^{-4.5}$	$10^{-4.7}$
RH-2*	Not done	$10^{-5.5}$	$10^{-4.4}$

* Titration after 2 passages in culture of the re-isolated RH strain. See text for details.
** By hemacytometer counts.

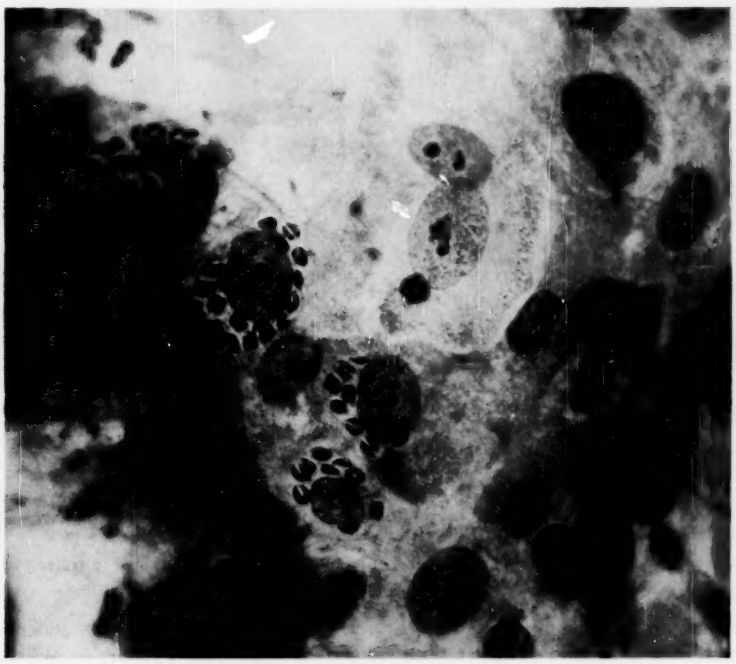

FIGURE 2. *Toxoplasma gondii* in epithelial cells derived from human foreskin tissue grown in roller culture. Coverslip preparation. Giemsa. 600×.

of *T. gondii* was made from infected mice by inoculation of mouse embryonic roller cultures with peritoneal fluid. Infected fluids from the second tissue culture passage of this isolate (RH-2) were titrated *in vivo* and *in vitro* simultaneously with those from the 22nd tissue culture passage of the original RH isolate. The results, as summarized in Table III, indicate that no significant alteration in infectivity occurred during the prolonged period of *in vitro* cultivation. Similar conclusions were reported by Meyer and de Oliveira (1943) who studied *Toxoplasma* under completely different conditions of *in vitro* cultivation.

C. In vitro *propagation of* Toxoplasma *in tissues of primates.*

The RH strain of *T. gondii* propagated *in vitro* in mouse tissues retained its capacity to infect human tissues. Thus, cultures of human skin (embryonic and adult) grown on coverslips were successfully infected with inocula derived from the 3rd, 8th, and 13th mouse embryo culture passages; and cultures of human uterine tissues were similarly infected with inocula from 3rd and 12th passages. The behavior of *Toxoplasma* in human tissue paralleled that in mouse tissue. Furthermore, all of the types of human cells present in the cultures appeared susceptible to infection and to the specific cytopathic effect of the parasite. For example, the organisms multiplied in squamous-like cells derived from human foreskin (Fig. 2).

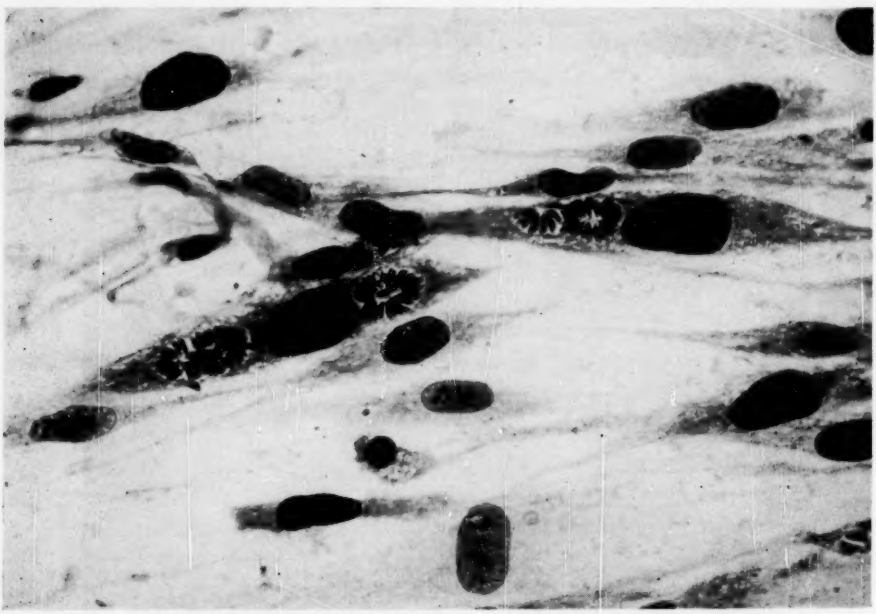

FIGURE 3. Rosettes of *Toxoplasma gondii* in monkey testicular cells grown in roller culture. Coverslip preparation. Giemsa. 600×.

Cultures of testicular tissue derived from the rhesus monkey were inoculated with fluids from the 21st mouse embryo culture passage of the RH strain, and these, too, supported growth of *T. gondii* (Fig. 3). Free organisms became apparent on the

4th day after inoculation and the course of events subsequently paralleled that in the mouse tissue cultures.

SUMMARY

The RH strain of *Toxoplasma gondii* was propagated for 23 serial passages and a second strain for 13 serial passages in roller cultures of mouse embryonic tissues. Prolonged *in vitro* cultivation of the organism did not significantly alter its infectivity as measured *in vivo* and *in vitro*.

The general sequence of multiplication of *T. gondii* and of the appearance of the specific cytopathic effects in roller cultures of mouse embryonic tissues was found to be relatively constant. However, it was shown that failure to renew the nutrient medium at intervals reduced the number of organisms produced in culture, and that the introduction of minimal inocula delayed their appearance by several days. Further experiments showed that: (a) *T. gondii* could be found intracellularly within 6 hours after inoculation; (b) during *in vitro* cultivation the organism probably multiplied intracellularly by binary fission; (c) *Toxoplasma* proliferated both in epithelial and fibroblastic cell types; and (d) all cells in inoculated cultures were ultimately destroyed. The elaboration of a stable soluble toxin by the organism in culture was not demonstrated.

A strain of *T. gondii* propagated in cultures of mouse embryonic tissues retained its capacity to multiply *in vitro* within cells derived from various human tissues and within cells originating from rhesus testicular tissues.

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TRANSPORT OF SPOROZOITES OF *EIMERIA NECATRIX* IN MACROPHAGES*

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According to the account by Tyzzer, Theiler, and Jones (1932), the sporozoites of *Eimeria necatrix* are liberated in greatest numbers in the middle portion of the small intestine of the chicken about an hour after ingestion of the oocysts, invade the tissue at once and become demonstrable in epithelial cells of the gland fundi of birds killed 1 hour after being fed the sporulated oocysts. They believed the sporozoites enter the intestinal glands before penetrating the epithelial cells (from the direction of the lumen of the gland) and that the sporozoites occasionally pass through the epithelium (of the gland fundi) and penetrate cells of the underlying tissue.

There are other accounts of sporozoite invasion of the lamina propria of chickens. In their account of *E. praecox,* Tyzzer, Theiler, and Jones state that in sections taken 8 hours after an infective feeding of oocysts, the sporozoites are found either deep in the epithelium of the villus or in the core of the villus, and that about as many sporozoites appear to pass through the epithelium as become established in it. Boles and Becker (1954) observed and figured sporozoites of *Eimeria brunetti* invading the cores of the villi but, like Tyzzer, considered that they did not undergo further development. Scholtyseck (1953) believed that the sporozoites of *E. tenella* pass from the crypts of Lieberkühn into fibrocytes of the underlying connective tissue, inside which cells they develop either in the mucosa or submucosa. Greven (1953) found the earliest stages of development of *E. tenella* in fibrocytes of the connective tissue of the mucosa. Hammond, Bowman, Davis, and Sims (1946) observed trophozoites of *E. bovis* as early as the uninucleate stage established in host cells located in the centers of the villi of the posterior half of the small intestine of cattle. In certain cases, early schizonts were seen inside endothelial cells in the endothelial lining of the lacteal, but the possibility that other unidentified cells of the reticular tissue also served as host cells could not be excluded. Lotze (1953) noted sporozoites of *E. arloingi* in the lamina propria and in the central lacteals of the small intestine of sheep at 7 and 9 days, and one sporozoite in an endothelial cell lining a central lacteal of a villus at 5 days.

Our observations on *Eimeria necatrix* in chickens indicate that the sporozoites of this species normally invade the lamina propria of the small intestine before entering the glandular epithelium.

MATERIAL AND METHODS

The cultures of *E. necatrix* employed for inoculating the chickens stemmed from a so-called pure culture isolated from a so-called mixed culture of *E. necatrix, E. acervulina,* and *E. mitis* by feeding 0.5% sulfaguanidine in the ration as advocated by Levine (1943). The hosts were either Hampshire Red or White Rocks from

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8 to 12 weeks of age, which were raised under circumstances that kept them from becoming accidentally infected with coccidia. In order to observe a satisfactory number of sporozoites and the early growth stages in the tissues, it was necessary to administer infective dosages of 15 to 25 million recently sporulated oocysts. Birds were sacrified at 12, 18, 24, and 48 hours after inoculation. Sections of the small intestine were put into alcoholic Bouin's fixative according to Duboscq-Brasil (Lee, 1950). The tissues were imbedded in paraffin, and sections were cut 5 microns in thickness and stained in Giemsa according to Shortt and Cooper (1948). Drawings were made with the aid of a camera lucida.

RESULTS

The first direct examinations of intestinal content made at 12 hours after the infective feeding revealed liberated sporozoites. Sections of tissues taken at the same time showed sporozoites entering and further invading the villi of the middle small intestine. In Figure 1 is shown a sporozoite that has penetrated the epithelium of a villus almost to the basement membrane. At 12 and 18 hours, there was extensive invasion of the lamina propria, and many of the sporozoites were observed to have become engulfed by macrophages (Figs. 3 and 4). A count of 100 sporozoites made at 18 hours indicated that 78% of them had already become enveloped by macrophages. Since no sporozoites were observed entering the openings of the intestinal glands at 12 or 18 hours, it is believed that the regular route of the initial invasion is through the epithelium of the villus, then through the lamina propria of the villus toward the muscularis mucosae. Enroute most of them become ingested by macrophages (Figs. 3 and 4).

At 18 hours, macrophages containing sporozoites were observed invading the glandular epithelium. These invasions were observed for the most part anywhere in the fundus epithelium, but there was some invasion of the duct of the gland, principally in that part adjoining the fundus. There were also invasions by sporozoites that seemed to be free, but it was impossible to tell whether they had ever been contained within macrophages. Figure 2 shows an epithelial cell of the gland fundus (upper left) invaded by an apparently free sporozoite, and next to it, on the right, one invaded by a macrophage containing a sporozoite. In Figure 5, there is another macrophage with its included sporozoite that has invaded an epithelial cell of the gland fundus.

It is evident that the sporozoite, whether contained within a macrophage or free in the lamina propria, enters the epithelium from the lamina propria. The invaded cells leave the position they occupy in the intact epithelium, become distorted in outline, increase in size, and move toward the gland lumen; and, as they do so, their nuclei enlarge very much and move distally ahead of the invading sporozoite (Figs. 2 and 5). At 24 and 48 hours, sporozoites not in macrophages were occasionally observed in the gland lumen, mostly in the fundus, but some of these were attached to the epithelium by means of tenuous strands of protoplasm proceeding from the cells. Such sporozoites probably entered the lumen of the gland by piercing the gland epithelium, for none was observed in the distal part of the duct of the glands.

Enveloping macrophages were not discernible by the time the sporozoite has rounded up and grown to the point where it exhibits a greatly enlarged nucleus.

Figure 6 shows a section of such a sporozoite (upper left), a section of a young schizont with a considerable number of nuclei and the refractile globule persisting from the sporozoite (lower left), and the enlarged nucleus of the host cell (upper right). In Figure 7 are seen 2 enlarged, displaced epithelial cells adjoining the gland lumen, each exhibiting an enlarged nucleus and parasitized by a growing multinucleate schizont.

DISCUSSION AND SUMMARY

Our account of the entrance of *E. necatrix* into the intestinal mucosa is in disagreement with that by Tyzzer, Theiler and Jones, who apparently assumed that the sporozoites enter the ducts of the intestinal glands and establish themselves inside the epithelial cells immediately after penetrating them from the gland lumen. They observed sporozoites in the tissue underlying the glandular epithelium, but considered they had arrived there after passing through the glandular epithelium.

Our studies indicate that the sporozoites of this species first enter the cells of the epithelium covering the villi of the small intestine, for the most part those in the middle small intestine. Then they pass through the epithelium into the lamina propria or core of the villus and migrate proximally in the direction of the muscularis mucosae. Enroute, most of them become engulfed by macrophages, though some of them seem to remain free. The epithelial cells of the fundus of the intestinal gland are invaded by the transporting macrophages. During or after the process of invading the epithelial cells, the macrophages appear to disintegrate, though a certain number of sporozoites not enveloped in macrophages can be found within the epithelial cells or free in the gland lumen. There is no evidence of digestion or other untoward effect of the macrophage on the sporozoite.

The introductory review has brought out that it is not unusual for sporozoites of *Eimeria* to penetrate into the connective tissue coat of the mucosa. An additional reason for believing the sporozoites would be inclined to enter by way of the villi rather than the ducts of the glands might be the flow of mucus secreted from the glands. In fact, Rees (1929) employed the same argument in defense of his view that *Entamoeba histolytica* does not enter the mucosa of the kitten by way of the intestinal glands. Failure to find sporozoites in the gland epithelium at 12 hours after feeding the infective oocysts, though they were present in the content of the intestine and in the villar epithelium and core at that time, is, however, the best evidence that entrance is not made via the openings of the glands. Admittedly, as stated previously, our infective doses of sporulated oocysts were very heavy, probably heavier than those employed by previous investigators.

It is well known that in sections of the intestinal epithelium lymphoid cells are often seen escaping into the lumen. It is possible that the macrophages with their included sporozoites are in the process of being similarly excreted. Within the gland epithelium, however, the intrinsic potentialities of the parasite for growth are stimulated, and it starts its development, leaving the transporting macrophage to its fate. The stimulus for the transformation of a sporozoite into a trophozoite is lacking in the lamina propria.

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EXPLANATION OF PLATE

Earliest endogenous stages of *Eimeria necatrix* in small intestine of chicken. x ca 3250. (Script g, refractile globule of sporozoite; l, location of lamina propria; m, macrophage enveloping sporozoite; n, nucleus of epithelial cell.)

FIGURE 1. Portion of epithelium near tip of villus showing sporozoite having penetrated almost to basal membrane at 12 hours after inoculation.

FIGURE 2. Sporozoite inside two adjoining epithelial cells in fundus of gland at 18 hours; right, sporozoite within macrophage; left, sporozoite without evidence of an enveloping macrophage.

FIGURE 3. Macrophage in lamina propria enclosing sporozoite, at 18 hours.

FIGURE 4. Macrophage in lamina propria enclosing two sporozoites, at 18 hours.

FIGURE 5. Cell from glandular epithelium invaded by macrophage with included sporozoite, at 18 hours.

FIGURE 6. Cell of glandular epithelium at 48 hours parasitized by (upper) growing sporozoite in cross section and (lower) young first generation schizont.

FIGURE 7. Portion of glandular epithelium at 48 hours with two dislocated and enlarged cells parasitized by (right) younger schizont and (left) more advanced schizont.

RESEARCH NOTE

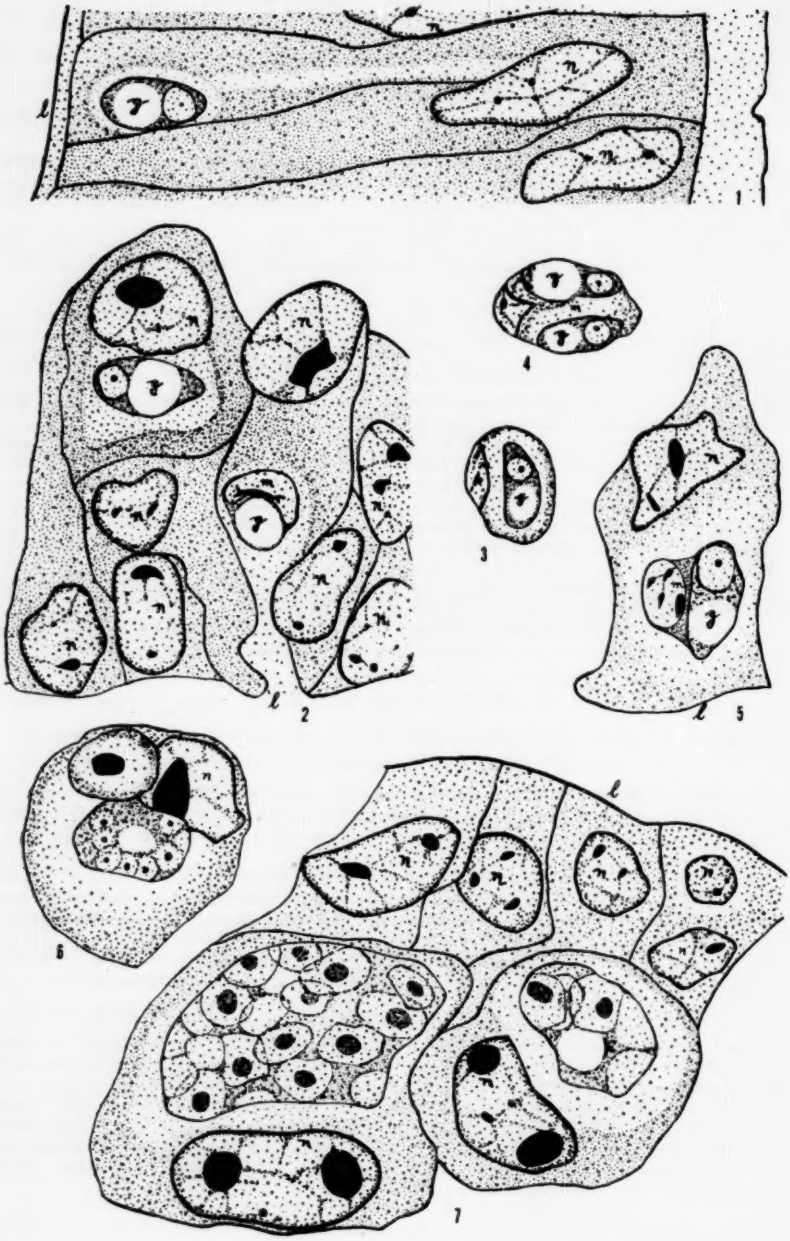
THE USE OF MYCOSTATIN (NYSTATIN) IN ISOLATION OF AXENIC CULTURES OF TRICHOMONADS.

The presence of fungi in the mouth of birds often makes the isolation of axenic cultures of *Trichomonas gallinae* difficult, if not impossible. Also, accidental contamination of bacteria-free cultures of other trichomonads, for instance *Trichomonas vaginalis*, results, almost invariably, in the loss of the strains.

The incorporation of 300 gamma/ml. of Mycostatin (Squibb) into a suitable medium (CPLM, simplified trypticase, or BBL fluid thioglycollate) containing 5% normal horse serum as well as 5,000 units of penicillin, 2,000 gamma of dihydrostreptomycin, and, in some instances, 250 gamma of chloramphenicol per ml. facilitated the isolation of several strains of *T. gallinae* directly from the mouth of pigeons. The addition of similar dosages of Mycostatin to cultures of *T. gallinae* or *T. vaginalis*, accidentally contaminated with fungi, cleared these cultures in 24 to 72 hours.

No strain of *T. gallinae*, including the well-known Jones' Barn strain of Stabler, or of *T. vaginalis*, including the original Kupferberg strain, appeared to be affected by the treatment. The rate of growth of the several strains on various media, and the morphology of the flagellates remained unchanged. Furthermore, the strains exhibited the same amount of pathogenicity to mice and tissue cultures before and after exposure to Mycostatin. Since some of the strains were isolated with the aid of Mycostatin, the last statement is based primarily on those which were isolated without the use of this drug.—BRONISLAW M. HONIGBERG, *Department of Zoology, University of Massachusetts, Amherst.* (This investigation was supported by a Public Health Service Research Grant (E-742).)

PLATE I



STUDIES ON THE FEEDING HABITS OF THREE SPECIES OF LAELAPTID MITES

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The epidemiology of haemorrhagic fever in Korea is extremely similar to that of scrub typhus, a disease that is known to be carried by mites, *Trombicula akamushi* Brumpt, 1910, and related species. Traub *et al* (1954) on the basis of extensive studies of possible vectors of haemorrhagic fever, concluded that the most probable vector was *Trombicula pallida* (Nagayo *et al*, 1919). However, they seriously considered *Laelaps jettmari* Vitzthum, 1930 as a vector because of a report that the disease had been recovered from it. They found that knowledge about the habits of *L. jettmari* and its relatives was extremely meager. The only evidence that these mites were parasitic was the fact that they were usually found associated with rodents, and occasionally a specimen was found that had red fluid (blood) in its gut. Vitzthum (1930) in a study of mites as potential vectors of plague concluded that *L. jettmari* and its relatives would not feed on man. No real knowledge concerning the feeding habits of mites of the family Laelaptidae was available. Lt. Col. Traub therefore recommended that the Medical Research and Development Board, Office of the Surgeon General, Department of the Army contract[2] with the University of Maryland to investigate the habits of laelaptid and related mites. The results of these investigations, in so far as they relate to feeding of laelaptid mites, are presented here.

Although the behavior of laelaptid mites was relatively unknown, the related dermanyssid mites were reported to feed on their natural hosts and on man (Baker *et al*, 1956). The techniques for investigating the feeding behavior of the laelaptid mites were developed by extensive studies of two species of dermanyssid mites, *Ornithonyssus bacoti* (Hirst, 1913) and *Ornithonyssus bursa* Berlese, 1888), (Cross and Wharton 1954, Cross, 1954). The data obtained from investigation of *O. bacoti*, the tropical rat mite, and *O. bursa*, the tropical fowl mite, as well as data reported in this paper, were used by Cross (1955) in a study of host specificity of blood-sucking mites.

MATERIALS AND METHODS

Three species of laelaptid mites: *Laelaps nuttali* Hirst, 1915; *Echinolaelaps echidninus* Berlese, 1887; and *Haemolaelaps glasgowi* (Ewing, 1925) were maintained in culture (Cross and Wharton, 1954) in the laboratory. The original sources of these 3 species were: *L. nuttali* from Lubbock, Texas, provided by Dr. R. W. Strandtmann; *E. echidninus* from Savannah, Georgia; and *H. glasgowi* from College Park, Maryland. Mites for experimental study were aspirated from the cultures into glass tubes approximately 3 inches in length and 0.25 inch in diameter. The mites were allowed to feed either on the skin of the host after the method of Cross and Wharton (1954) or on a bolting silk membrane after the method of

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Cross (1954). In order to reduce variability resulting from differences in age and nutritional states of mites, comparative tests were always run simultaneously on mites from a single culture that had been starved for at least 1 day.

Attempts were made to feed mites on the intact skin of chickens (*Gallus domesticus* L.) and men (*Homo sapiens* L.). Mites were also exposed to human skin that was abraded by removing the upper layers with a sharp razor until blood became visible, but before droplets could be formed. Various mixtures of blood and blood fractions were used in connection with the bolting silk membrane. Blood was taken into a 1-cc tuberculin syringe that had previously been flushed with a 0.1% solution of heparin in physiological saline. Whole blood was diluted with physiological saline to decrease its viscosity before it was placed on the bolting silk membrane. Blood cells were separated from the plasma by centrifuging. The cells were then washed twice in physiological saline. After the final washing they were suspended in enough physiological saline to make a 50% suspension. Serum was obtained by allowing whole blood to clot. Four drops of a 1% solution of Evan's Blue were added to each cc of plasma or serum. The blue color made it easy to differentiate between fed and unfed mites.

The effects of temperature and humidity on the feeding of mites were tested by allowing the mites to feed on heparinized whole blood diluted with physiological saline. Ten mites of a given stage were exposed to the blood. The number that were filled with blood at the end of a 30-minute exposure were then counted. Temperatures were measured with a Rauh surface pyrometer or a thermometer. Humidities were measured with an Aminco-Dunmore Hygrometer. Variable humidities were obtained by using an Aminco-Aire Climatizer. Both temperature and humidity were maintained during these tests. Controls for the humidity tests were run in an incubator where the humidity remained close to 22% R. H. even though the temperature was adjusted to the temperature in the climatizer. In tests on the effect of temperature, a small box that was heated by circulating hot water was used. The humidity in this box fluctuated between 16 and 35% R. H. In all tests, the mites were exposed to the experimental conditions for 15 minutes before the tests were begun.

The results of the tests were expressed in absolute terms or in comparative terms as a difference from a standard. When mean differences were determined, the standard error was also calculated to evaluate their significance.

RESULTS

Temperature appeared to have a marked effect on feeding and its effect was different in the case of each species (Table I). *E. echidninus* fed well at high (95–105° F) and low (65–70° F) temperatures but its feeding was depressed at intermediate ranges (75–85° F). *Laelaps nuttali* fed best at temperatures above 85° F. *Haemolaelaps glasgowi* fed well at temperatures above 75° F, but its rate was considerably curtailed at temperatures between 65° and 75° F. Tests were made on both nymphs and adults of *E. echidninus* and *L. nuttali*. Temperature seemed to affect the nymphs and adults in the same way.

More mites of all 3 species fed at a low relative humidity (22%) at a temperature of 95° F than fed at higher humidities (Table II). With 2 exceptions, however, no significant differences between feeding rates at low and high relative humid-

ities were found. *E. echidninus* adults and nymphs had a significantly lower feeding rate at 96% R. H. than at 22% R. H.

All 3 species of mites fed readily on the whole blood of bird and rodent as well as on the blood fractions (Table III). Nymphs of *E. echidninus* and *L. nuttali* fed as well or better than did the adults. No definite preference for a particular kind of blood or for a particular blood fraction was noted.

TABLE I— *Difference in feeding of* E. echidninus, L. nuttali *and* H. glasgowi *on chicken blood at low relative humidities (16-35%) at different temperatures*

°F	105	95*	85	75-81	65-70	105	95*	85	75-78	65-70
				E. echidninus adults					*E. echidninus* nymphs	
	-2	10	-1	0	-1	0	10	-5	0	0
	-2	10	-5	-5	-1	0	10	-7	-5	0
	2	8	-1	-1	1	0	10	-1	-6	0
	-1	10	-6	0	0	1	9	1	-5	1
	0	10	-	-5	-2	1	9	-	-9	-7
	1	8	-	-5	-2	2	8	-	-6	-4
	-	7	-4	-3	-1	-	-	-	-	-
	-	5	-5	-1	-4	-	-	-	-	-
	-	6	-1	-3	-4	-	-	-	-	-
	-	8	-7	-7	-6	-	-	-	-	-
	-	3	0	-3	3	-	-	-	-	-
Total	-2	94	-30	-29	-17	4	56	-12	-31	-10
Mean	-0.3	8.5	-3.3†	-2.6†	-1.5	0.7	9.3	-3	-5.2‡	-1.7
				Laelaps nuttali adults					*Laelaps nuttali* nymphs	
	2	7	-3	-5	-4	2	5	0	-2	-5
	-1	9	-5	-1	-7	0	5	0	-3	-5
	0	9	1	-8	-3	0	7	-2	-3	-6
	0	9	1	-8	-3	0	5	-3	1	-3
	0	8	-6	1	-1	1	8	-	-5	-7
	4	5	-	3	-0	-3	10	-	-5	-9
	-5	10	-	-8	-8					
Total	0	48	-13	-18	-23	0	40	-5	-17	-35
Mean	0	8	-3.3†	-3.0	-4.0‡	0	6.7	-1.3	-3.0‡	-6.0‡
				H. glasgowi adults						
	-	10	-3	-5	-8					
	-	10	-5	-	-6					
	-	7	3	-1	-3					
	-	2	5	5	2					
	-	10	-4	-3	-6					
	-	6	4	3	-3					
Total	-	45	0	-1	-24					
Mean	-	7.5	0	-0.2	-4.0‡					

*95°F. standard for comparison. Numbers are actual number of mites that fed in each tube of 10 mites during the test.

†Difference significant at the 0.05 level.

‡Difference significant at the 0.01 level.

None of the species was seen to feed through the undamaged skin of a host although they were exposed to the host under conditions of temperature and humidity that were conducive to feeding, whereas controls under the same conditions fed readily on blood (Table IV). Two specimens of 20 *E. echidninus* did feed on a rat whose skin was not purposely abraded, but this rat was not known to have an undamaged skin. On the other hand, specimens of all 3 species fed readily on abraded human skin. They did not merely lap fluid from the surface of the skin, but they inserted their mouthparts into the dermis.

TABLE II — *Difference in feeding of* E. echidninus, L. nuttali *and* H. glasgowi *on chicken blood at 95° F at different relative humidities compared with a standard at 22% R. H.*

	E. echidninus adults		*L. nuttali* adults		*H. glasgowi* adults
	R. H. 96%	R. H. 83%	R. H. 96%	R. H. 83%	R. H. 96%
	-2	-1	-9	-10	0
	-3	0	0	1	1
	-3	-2	-5	-3	0
	-5	1	1	-3	-1
	-3	0	-9	1	-7
	-4	-3	-1	0	-2
Total	-20	-5	-23	-14	-2
Mean	-3.3*	-0.1	-3.8	-2.3	0
	nymphs		nymphs		0
	-1	0	-5	-2	0
	-4	0	0	-2	0
	-5	0	0	-3	0
	-4	0	1	-1	1
	-7	0	-9	-3	-1
	-6	0	-1	-3	1
					0
Total	-27	0	-14	-12	-10
Mean	-4.5*	0	-2.3	-2.0	-0.6

*Difference significant at the 0.01 level.

TABLE III — *Numbers of* E. echidninus, L. nuttali *and* H. glasgowi *that engorged on different fractions of different kinds of blood at 95° F and low relative humidities (16-35%). 20 mites were used in each test.*

Source	Whole Blood	Red Cells	Plasma	Serum
		Echinolaelaps echidninus adults		
chick	18	14	17	19
rat	15	18	14	16
mouse	13	17	17	16
		Echinolaelaps echidninus nymphs		
chick	18	16	18	16
rat	14	13	15	16
mouse	16	20	20	17
		Laelaps nuttali adults		
chick	3	4	8	8
rat	5	10	3	10
mouse	4	7	9	11
		Laelaps nuttali nymphs		
chick	12	10	6	9
rat	14	11	7	13
mouse	8	6	6	15
		Haemolaelaps glasgowi adults		
chick	13	15	16	15
mouse	18	15	11	15

TABLE IV — *Number of* E. echidninus, L. nuttali *and* H. glasgowi *fed through skin compared with standard. Numbers in parentheses are number of mites that fed on skin*

	Type of Skin			
	Rat	Chick	Human	Human Abraded
E. echidninus	-18 (2)	-20 (0)	-45 (0)	0 (10)
L. nuttali	-5 (0)	-5 (0)	-5 (0)	0 (10)
H. glasgowi		-	-9 (0)	-2 (8)

DISCUSSION

That *E. echidninus, L. nuttali,* and *H. glasgowi* are blood-sucking ectoparasites has been demonstrated. They can be readily cultured on small rodents from which they obtain blood. They differ from many ectoparasites in that they do not regularly penetrate the unbroken skin of their hosts. Even under proper conditions of temperature and humidity, they have not been seen to make an attempt to feed on the unbroken skin. Actually these mites appear to take little interest in their host. Even starved specimens will wander on and off a host apparently at random. They do, however, appear to be attracted to vertebrate blood or any of its components. It is probable that these mites depend upon other ectoparasites, accidents, or the scratching of the host itself to prepare the skin for them. Once the skin is broken, it is not unusual to see a number of these mites clustered about the break in the process of feeding.

It has also been demonstrated that these mites will bite man as readily as other hosts if an opportunity presents itself. Few men have a completely unbroken skin. It therefore is probable that contact of man with a large number of laelaptid mites will result in the man being bitten by at least a few of them. The fact that their bites are superimposed on other injuries is probably the reason that these mites have not previously been recognized as attacking man. Laelaptid mites can no longer be ignored as possible vectors of disease to man.

The only evidence that *L. jettmari* might be a vector of haemorrhagic fever in Korea was the report that inoculation of a suspension of 203 *L. jettmari* into a man had produced the disease (Traub *et al,* 1954). To this can now be added the fact that its relatives can and will bite man. All other evidence about *L. jettmari* such as its geographical and seasonal distribution suggests that it is not a vector, but in no way does it prove that it is not. *L. nuttali* has been considered (Cole and Koepke, 1946) as a possible vector of endemic typhus in Mobile, Alabama, as follows: "*L. hawaiiensis* (= *nuttali*), although less abundant and not known to attack man or to be infectible with typhus, also exhibits seasonal and local distributions which indicate that it should not be disregarded as a possible vector." The fact that *L. nuttali* will bite man under certain conditions suggests that the potentiality of this mite as a vector of endemic typhus should be evaluated.

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RESEARCH NOTE

ECTOPARASITIC ACARINA FROM SMALL MAMMALS OF THE PATUXENT REFUGE, BOWIE, MARYLAND

During the period from September, 1953 through November, 1955, 279 small mammals were taken by live or museum special snap traps from various areas of the Patuxent Wildlife Research Refuge near Bowie, Maryland. These mammals were: 130 wood mice, *Peromyscus leucopus noveboracensis* (Fischer, 1829); 101 meadow mice, *Microtus pennsylvanicus pennsylvanicus* (Ord, 1815); 8 pine mice, *Pitymys pinetorum scalopsoides* (Audubon and Bachman, 1841); 2 Carolina jumping mice, *Zapus hudsonicus americanus* (Barton, 1799); 21 house mice, *Mus musculus* Linneaus, 1758; 7 short-tailed shrews, *Blarina brevicauda brevicauda* (Say, 1823); 7 Maryland shrews, *Sorex cinereus frontinalis* Hollister, 1911; 2 least shrews, *Cryptotis parva parva* (Say, 1823); and 1 star-nosed mole, *Condylura cristata* (Linneaus, 1758).

A list of the mites and ticks collected from these mammals is given below. Following each acarine are the names of the mammals from which it was recovered.

FAMILY HAEMOGAMASIDAE: *Haemogamasus liponyssoides* (Ewing, 1925), *P. leucopus, M. pennsylvanicus, P. pinetorum, S. cinereus, B. brevicauda; Haemogamasus barberi* Ewing, 1925, *P. pinetorum; Haemogamasus alaskensis* Ewing, 1925, *M. pennsylvanicus.* FAMILY DERMANYSSIDAE: *Ornithonyssus bacoti* (Hirst, 1913), *P. leucopus, M. pennsylvanicus; Ornithonyssus bursa* (Berlese, 1888), *P. leucopus, M. pennsylvanicus; Ornithonyssus sylviarum* (Canestrini and Fanzago, 1877), *M. musculus; Dermanyssus* sp., *P. leucopus, M. pennsylvanicus; Myonyssus jamesoni* Ewing and Baker, 1947, *B. brevicauda; Hirstionyssus arcuatus* (Koch, 1839), *B. brevicauda, C. parva.* FAMILY LAELAPTIDAE: *Haemolaelaps glasgowi* (Ewing, 1925), *P. leucopus, M. pennsylvanicus, P. pinetorum, M. musculus, S. cinereus, B. brevicauda; Eulaelaps stabularis* (Koch, 1839), *M. pennsylvanicus; Laelaps alaskensis* Grant, 1947, *M. pennsylvanicus, S. cinereus; Laelaps kochi* (Koch, 1836), *P. leucopus, M. pennsylvanicus, P. pinetorum, B. brevicauda.* FAMILY IXODIDAE: *Dermacentor variabilis* (Say, 1821), *P. leucopus, M. pennsylvanicus.* FAMILY TROMBICULIDAE: *Euschöngastia peromysci* (Ewing, 1929), *P. leucopus; Euschöngastia setosa* Ewing, 1937, *P. leucopus; Euschöngastia* sp., *M. pennsylvanicus; Trombicula jonesae* Brennan, 1952, *P. leucopus; Trombicula (N.) whartoni* Ewing, 1929, *M. pennsylvanicus; Trombicula (E.) splendens* Ewing, 1913, *Z. hudsonicus; Walchia americana* Ewing, 1942, *P. leucopus.* FAMILY MYOBIIDAE: *Myobia simplex* Ewing, 1938, *B. brevicauda; Myobia musculi* (Schrank, 1781), *M. musculus; Protomyobia claparedei* (Poppe, 1896), *B. brevicauda; Radfordia subuliger* Ewing, 1938, *P. leucopus; Radfordia lemnina* (Koch, 1841), *M. pennsylvanicus, P. pinetorum.* FAMILY LISTROPHORIDAE: *Myocoptes* sp., *M. musculus, P. leucopus, M. pennsylvanicus; Listrophorus* sp., *P. leucopus, M. pennsylvanicus, P. pinetorum, B. brevicauda.*

The kinds of Acarina collected from these mammals do not differ from those reported from the same species of mammals in Delaware (MacCreary, 1945, J. Econ. Ent. **38** (1): 126–127) and in West Virginia (Wilson, 1943, J. Mamm. **34** (1): 102; Wilson, 1945, J. Mamm. **36** (2): 200). However, herein reported are new host and locality records for *W. americana* Ewing and *T. jonesae* Brennan.

The author wishes to acknowledge Mrs. L. Stickle for trapping some of the mammals, Dr. R. W. Strandtmann for aid in identifying dermanyssid mites, and C. M. Clifford who identified the Ixodidae. This project was done with the cooperation of the Fish and Wildlife Service, United States Department of Interior, and was supported by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army, under Contract No. DA-49-007-MD-501 with the University of Maryland.—ROGER O. DRUMMOND, *Department of Zoology, University of Maryland; present address, U. S. Dept. Agriculture, Entomology Research Laboratory, P. O. Box 232, Kerrville, Texas.*

EXPERIMENTAL FEEDING OF THE MITE *BREVISTERNA UTAHENSIS* (ACARINA: HAEMOGAMASIDAE)

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During a seasonal study of the arthropod fauna associated with the nests of the desert wood rat, *Neotoma lepida lepida* Thomas, in Utah, large numbers of the parasitic mite *Brevisterna utahensis* (Ewing), were collected. In order to maintain these mites for life-history and experimental transmission studies in the laboratory, it was necessary to devise a method whereby they could feed periodically on blood. The method initially employed was similar to that used by Hopla (1951). The wood rat and the Ord kangaroo rat, *Dipodomys ordii pallidus* Durrant and Setzer, were utilized as the laboratory animals upon which the mites fed. The tip of each rat's tail was cut off and the mites readily fed upon the exposed blood, some engorging in less than 1 minute. However, the disadvantages of this method were that (1) it required considerable time and effort to set up the apparatus for feeding ,(2) the simultaneous feeding of large numbers of mites required a proportionately large number of host animals, (3) an excessive amount of bleeding often entrapped many of the mites and caused their death, and (4) it was difficult to control the mites while feeding.

The preliminary experiments indicated that *B. utahensis*, a common inhabitant of nests of the desert wood rat in Utah, will feed on blood of an animal other than its usual host, the wood rat. In subsequent experiments, several mites also fed on blood serum from a wood rat. This serum, which had been frozen for approximately 6 months, was warmed to room temperature before feeding. These observations indicated that it might be possible to use stored blood for feeding mites and eliminate the need for live animals. Although Cross and Wharton (1954), Cross (1954), and Strandtmann (correspondence) used techniques involving the use of stored blood, the technique used in these experiments was developed independently and without knowledge of the existence of the very similar techniques of these contemporary workers. These methods of feeding parasitic mites successfully overcome the disadvantages of the Hopla method.

METHODS

Blood was collected by heart puncture from native rodents of 5 species: *Citellus leucurus leucurus* (Merriam), the antelope ground squirrel; *Perognathus formosus incolatus* (Hall), the long-tailed pocket mouse; *Peromyscus maniculatus sonoriensis* (LeConte), the deer mouse; and the desert wood rat and Ord kangaroo rat. Each of 6 blood samples from the ground squirrel, kangaroo rat, wood rat, pocket mouse, and deer mouse, was treated with a 2% solution of sodium citrate (18 parts of citrate per 100 parts of blood) and stored at 4°C until used for feeding. One sample of *C. leucurus* blood was treated with a 2% solution of ammonium potassium oxalate* (10 parts of oxalate per 100 parts of blood) and stored at 4°C. Two blood samples each of *D. ordii* and *C. leucurus* were frozen without the addition of anticoagulants. The clots were broken up as much as possible, and repeated freezing

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*1.2 gm. ammonium oxalate and 0.8 gm. potassium oxalate in 100 ml distilled water.

and thawing caused hemolysis of the blood. One sample of citrated, whole blood of man (1 part citric acid dextrose solution B per 4 parts of blood) was also used. In all cases the blood was allowed to warm to room temperature before experimental feedings were begun. From 17 to 40 mites were fed individually on each kind of blood, and a total of 226 mites was used in the feeding experiment. In addition, 2 series of control experiments were run wherein 57 mites (fed-controls) were permitted to feed on fresh, whole blood from the tails of kangaroo rats and wood rats as described above, and 39 mites (unfed-controls) received no food throughout the experiment.

Deutonymphal and adult mites of both sexes used were kept in glass tubes 8 mm × 75 mm, which were stoppered at both ends with cotton plugs wrapped in cheese cloth. Between feedings the mites were kept at a relative humidity of 81% at room temperature.

The feeding apparatus consisted of a glass tube and pipette fitted together in the following manner. The glass tube containing mites was held vertically and gently tapped with the finger. This caused the mites to fall to the lower end of the tube. A one-hole rubber stopper was substituted for a cotton plug in one end of the tube, and the small end of a standard 3-inch pipette (medicine dropper), from which the bulb had been removed, was inserted into the hole in the rubber stopper. After a drop of blood was placed into the pipette, gentle tapping caused the blood to flow to the extreme tip of the tapered end. The cotton plug in the opposite end of the tube was pushed gently toward the blood until it was about 1/16 inch from the pipette. Most of the mites fed within 10 minutes, but the apparatus was left assembled for 30 minutes in order to accommodate stragglers. After feeding, the mites usually congregated on the cotton plug which was then pulled back to the opposite end of the tube, the pipette was removed, and the rubber stopper replaced with a cotton plug.

The experiments were conducted for 4 weeks, during which time the mites were offered blood at intervals of 1 to 5 days.

RESULTS

When given the opportunity, at least 40% of the mites fed. The variation between the numbers that fed on different kinds of blood was slight except in the case of the oxalated blood of the ground squirrel in which, with 1 exception, less than 70% of the mites fed. In most other cases, considerably more than 70% fed.

Ninety percent of the unfed-controls died by the 14th day (Fig. 1), and all were dead by the 21st day. Only 16% of the fed-controls were dead by the 8th day; an additional 10% had died by the 21st day. Seventy-four percent were still alive at the end of 28 days.

The survival of mites fed on citrated blood was approximately equal to that of the fed-controls. Eighteen percent died by the 8th day. By the 28th day, 72% were still alive except in the case of those that fed on blood of the pocket mouse. Of these latter, only 41% survived.

Of the mites fed on oxalated blood, 33% were dead by the 12th day. At the end of 28 days, 61% were still alive.

Of those fed on frozen, untreated blood, 13% died during the first 5 days, and an additional 5% by the 12th day. There were no deaths thereafter.

DISCUSSION

These experiments show that mites of the species *Brevisterna utahensis* are able to survive on any one of several kinds of blood preserved by anticoagulants or by freezing. Blood from man and 5 species of rodents apparently was accepted with equal readiness. There appeared to be little preference for blood treated in any one particular manner. However, there was some indication that the oxalated blood from the ground squirrel was the least acceptable. By the 14th day, 90% of the unfed-controls were dead, and by the 21st day, all unfed-control mites were dead. An average of 74% of all other mites were still alive at the end of 28 days (Fig. 1).

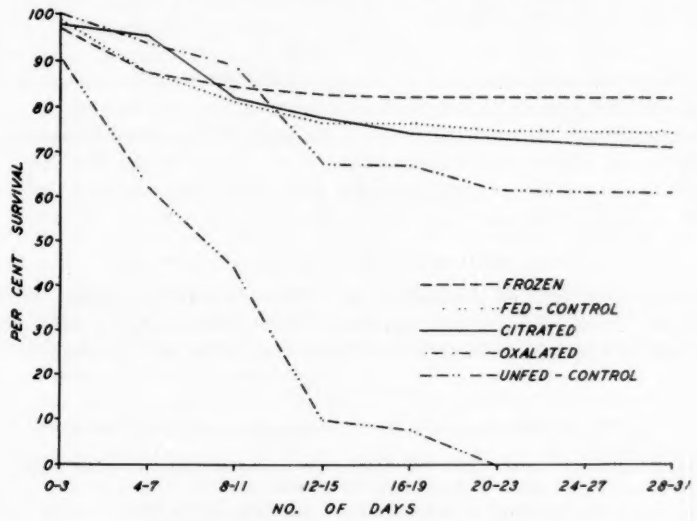

FIGURE 1. Survival of mites fed on blood.

A much higher percentage of the mites in the feeding experiments and the fed-controls died by the 13th day than during the remainder of the experiments. Of the 75 mites that died during the experiment, 79% died before the 13th day. This may indicate that those mites dying prior to the 13th day may not have fed and presumably died from starvation. If this were the case, it may be assumed that the majority of those that did feed survived. Thus it appears that the blood and small amounts of anticoagulants used in these experiments did not seriously affect the mites, with the possible exception of blood from the pocket mouse, which was inadvertently citrated too heavily.

Not all of the mites fed each time that they were given an opportunity. They may not have been hungry because of a recent feeding, or may have had some other physiological or sensory reaction to the different blood or to the anticoagulant. In many cases, however, some mites fed slightly each time blood was offered even though they were partially engorged from a previous feeding.

Although the immature stages of many species have feeding preferences or habits different from those of adults, no differences were observed between the deutonymphs and adults of *B. utahensis*. Approximately equal numbers of both

stages survived. Several of the deutonymphs molted to adults, and many females produced eggs which hatched, and the larvae and protonymphs were reared through to the deutonymphal stage.

Whether or not this method of feeding will prove satisfactory for mites of other species is not known. This method may be useful in further experiments dealing with (1) the artificial feeding of parasitic mites other than *B. utahensis;* (2) mite preference or acceptability of blood from different kinds of animals; (3) the use of artificial media and organic matter other than blood for feeding mites; (4) the effects of temperature and humidity on feeding efficiency; and (5) the experimental transmission of pathogenic and non-pathogenic organisms including viruses, bacteria, protozoa, and helminths.

SUMMARY

Deutonymphal and adult mites of the species *Brevisterna utahensis* were fed on citrated, oxalated, and untreated blood of rodents of 5 species and man. Seventy-four percent of both the artificially fed and the naturally fed control mites survived a 28-day period, whereas unfed-control mites were all dead by the 21st day. Blood of all kinds used apparently was accepted with equal readiness, and the rates of survival of mites fed on each type varied from 61% to 82%.

ACKNOWLEDGMENT

We wish to express our gratitude to the former Director, Dr. Angus M. Woodbury, to Mr. Harold Egoscue for supplying the host animals, and to other members of the staff of Ecological Research for helpful suggestions and assistance.

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RESEARCH NOTE

PARASITES OF THE CARP, *CYPRINUS CARPIO* L. IN LAKE TEXOMA, OKLAHOMA

A study of the endo- and ectoparasites of the German carp from Lake Texoma was undertaken during the summer of 1955. This report is part of the investigations of fish parasites carried out at the University of Oklahoma Biological Station. The author is indebted to Dr. J. Teague Self for supervision of research, to Dr. John D. Mizelle for confirmation of identity of the Monogenea, and to Mr. Leon Harney and Mr. Virgil Dowell for aid in collection of hosts.

Fifty-nine fish were collected and autopsied near the Buncombe Creek inlet of the lake from 23 June to 17 September. Over 90% of these were infected by ectoparasites while only 3.4% had endoparasites. *Camallanus ancylodirus* Ward and Magath (Nematoda: Camallanidae) was the only intestinal helminth observed. Two fish were infected with a total of 3 nematodes. *Dactylogyrus extensus* Van Cleave and Mueller (Monogenea: Dactylogyridae) was found on the gills of 70% of the carp examined. The maximum number on a single host was 16, and the average infection was 4 worms. The present specimens agree with the redescription as given by Mizelle and Klucka (1953, Amer. Midl. Nat. **49**: 720–733). A single *Argulus flavescens* Wilson (Crustacea: Argulidae) was taken from the branchial cavity of 1 host. Several copepods probably belonging to the Family Ergasilidae were found in the branchial cavities of 93% of the hosts examined.—LARRY S. ROBERTS, *Department of Zoology, University of Illinois, Urbana, Illinois.*

THE PATHOGENIC EFFECT OF *TRYPANOSOMA DUTTONI* IN HOSTS UNDER STRESS CONDITIONS

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Parasites of the *Trypanosoma lewisi* group are usually regarded as non-pathogenic. Theoretically no parasite can be totally without adverse effect on its host, but the effect may be so slight as to escape notice. It is possible that such a parasite may have more serious effects when the host is subjected to stress conditions. The present experiment was designed to test this hypothesis under laboratory conditions.

Various members of the staff of the University of British Columbia assisted in planning and carrying out the work. Special thanks are due to Dr. A. J. Wood and the Department of Animal Husbandry for providing the necessary facilities. The work received financial support from the University Research Committee.

MATERIALS AND METHODS

Parasitized animals and controls were subjected to stress conditions, and their survival and weight change were observed. The stress conditions were chosen to duplicate natural stresses as closely as possible. An important stress period for animals in the northern regions of the world is winter, and for most mammals the most important stresses are low environmental temperature and inadequate food supply. These were the stresses applied here.

The host used was the laboratory mouse. The parasite was *Trypanosoma duttoni*, a member of the *T. lewisi* group which is specific to the house mouse. Infections were established by intraperitoneal injection of blood from a reservoir mouse. All of the injected mice became infected.

After the infection was established, half of the mice were kept at room temperature (66–71°F), while the other half were placed in a cold room (38–47°F). All animals were caged individually, and no shelter was provided. The relative humidity in the cold room was much higher than that in the warm room.

Food was supplied in the form of pellets of a standard balanced mouse ration. Much of it was wasted by all the mice. The *ad libitum* food consumption of each mouse was determined in both warm- and cold-room groups. Half of the mice in each group were then restricted to one-half of their *ad libitum* consumption, while the remainder continued to receive a surplus of food. Individual caging assured proper distribution of rations. Water was available at all times.

A complete set of controls was used, giving a total of 8 groups (Table I). Each group consisted of 15 mice—6 male and 9 female. All mice were from a local inbred line. Age and weight were equalized among the groups as much as possible.

RESULTS

The first deaths occurred on the third day after the mice were reduced to half rations, and the experiment was ended on the nineteenth day.

It is apparent from the raw data that each of the conditions affected survival,

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TABLE I.—*Survival and weight change of mice*

Group			Survival over 19-day period			% Weight change
			No. of survivors	Mean days survived	Mean days dead	
Full Ration	Warm room	non-parasitized	15	19.0	0	+14
		parasitized	15	19.0	0	+ 7
	Cold room	non-parasitized	15[1]	19.0	0	+15
		parasitized	15	19.0	0	+ 7
Half Ration	Warm room	non-parasitized	12	17.7	1.3	−20
		parasitized	8	16.5	2.5	−16
	Cold room	non-parasitized	0	6.7	12.3	−19
		parasitized	0	4.6	14.4	−24

[1] One clearly aberrant non-parasitized mouse on full rations in the cold room died on the third day of the experiment and is omitted from these data.

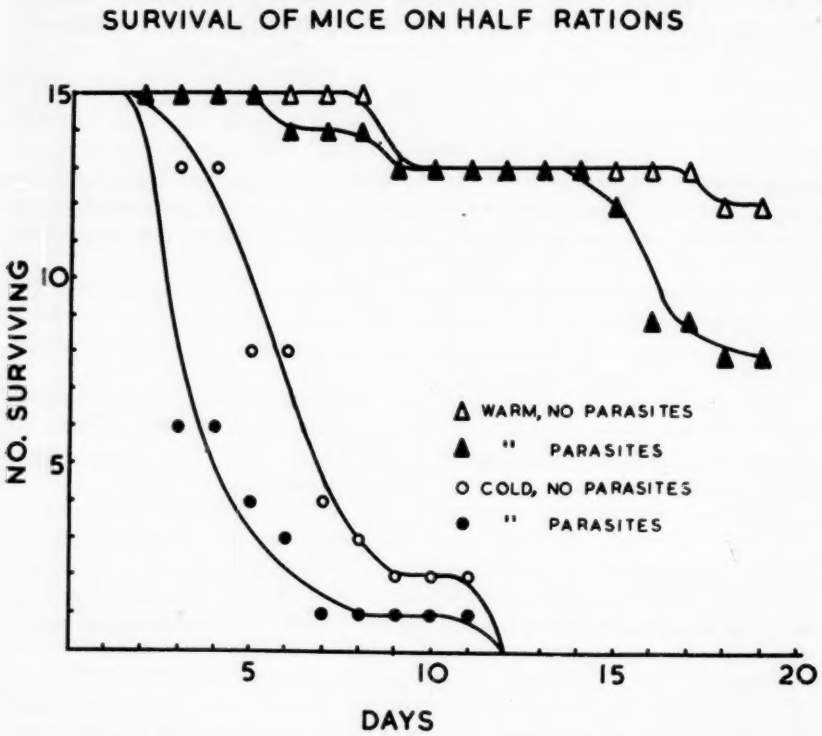

FIGURE 1. Survival of mice on half rations. Curves have been fitted by sight.

but each to a different degree (Table I). As none of the full-ration mice died, while some mortality was observed in all groups on half rations, it is clear that starvation was the most important lethal factor.

A survivorship curve for each of the half-ration groups is plotted in Figure 1. It will be seen that mice in both of the cold-room groups died much sooner than did

the comparable groups in the warm room. In both rooms the parasitized mice died appreciably sooner than the non-parasitized.

The dieoff of the 2 cold-room groups can be divided into 4 periods: (1) a brief premortal period during which all of the mice still were able to cope with the stresses imposed on them, (2) a period of rapid dieoff, lasting until almost all the mice were dead, (3) a period during which one or two unusually hardy mice continued to survive, and (4) a brief period in which the last mice died. Both groups entered Period 2 on the third day of the experiment. The parasitized group entered Period 3 on the seventh day, the non-parasitized on the ninth day. The last mice of both groups died on the twelfth day. The survival difference between the 2 groups came in Period 2. During this period the non-parasitized mice died at an almost even rate, while the parasitized mice died more rapidly at first and more slowly toward the end of the period.

It was not possible to continue the experiment long enough for all the half-ration mice in the warm room to die. Two animals in both groups died at about one week, but the remainder of the animals survived for at least 2 weeks. A period of sustained dieoff began in the parasitized mice on the fifteeenth day and by the end of the experiment 7 animals had died. Only 3 of the non-parasitized mice died. The data are not complete enough to permit comparison with the cold-room groups, but they suggest that the complete survivorship curves would be similar, though greatly extended. The parasitized group seems to have entered Period 2 on the fifteenth day.

Parasitized and non-parasitized groups were compared by means of the "t" test. The survival difference between the 2 groups in the cold room was found to be significant, but too few animals died in the warm room to make such a comparison possible. If only the data from the periods of rapid dieoff are used, the differences are significant in both cases.

Most of the mice in each group remained in good condition until near death. Mice in the cold room ruffled their fur but remained active. Previous workers had reported marked edema and gangrene of the extremities in the cold (Gilson, 1950), but there was little indication of this here. The slight edema that did appear had largely cleared up by the end of the first week.

The most striking feature seen at the post mortem examinations was extensive gastric and intestinal hemorrhage. This occurred in the half-ration mice at both temperatures. In most of the mice the stomach and intestine were flabby and transparent, and contained considerable amounts of granular, coffee-colored blood. The adrenals of the cold-room mice were inflamed at the time of death, but those of the controls were not. There was no indication of a pneumonic condition. Mice which died early in the experiment still had normal fat reserves, while those which died late had completely exhausted their reserves. The last mice to die had small, dark livers. No difference was noted in post mortem appearance of parasitized and non-parasitized mice.

The mice in each of the half-ration groups lost an average of approximately 20% of their initial weight before dying, with no clear indication of any influence from temperature or parasite. The full-ration mice gained weight. Those in the cold room gained as much as those in the warm room, but the parasitized animals averaged only half as much percentage gain (7%) as the non-parasitized (14%)

(Table I). There is too much intra-group variation for the data to show statistical significance, but the data from both rooms strongly suggest that the infection actually impeded growth in these animals.

DISCUSSION

The trypanosome infection appears to have had a definite effect on the host animals. The partially starved mice which were parasitized tended to die sooner than did those which were not parasitized. The full-ration mice which were parasitized averaged only half as much weight gain as did controls. In all 4 pairs of groups the non-parasitized mice appear to have had an advantage over the parasitized. In no one pair was the difference conclusive, but the consistency of the results in all 4 pairs lends weight to the hypothesis.

The mechanism of this effect of the parasite is not clear. It would be expected that if both groups had received the same amount of food those exposed to cold would have died first, because their energy requirements would be greater. In this case, however, both groups received the same proportion of their initial voluntary consumption. It is possible that their requirements increased later in the experiment.

Kolodny (1940) found that *T. cruzi* infections were much more severe in rats kept at 40–45°F than in rats at 70–74°F. Rats kept at the lower temperature invariably died, while those at room temperature usually survived. In the present work no mice died unless starved, but *T. duttoni* is much less pathogenic than *T. cruzi*.

It is known that fasting animals first deplete their carbohydrate stores, then utilize their fat reserves, and finally, in the pre-mortal period, rely largely on body protein (Chambers, 1938). The transition from carbohydrate to fat metabolism and from fat to protein metabolism requires a certain amount of time, and it is possible that the cold-room mice exhausted their carbohydrate reserves before they had time to change to fat metabolism, and so had no energy reserves which they were capable of utilizing. This is supported by the fact that animals which died early in the experiment still had large quantities of fat, while those which died late had none.

Quay, (1955), working with an unnamed trypanosome in lemmings (*Dicrostonyx*) found that anemia was the only marked effect of the infection. The most heavily infected individual had only half as many erythrocytes as controls. There was no effect on the leucocytes. He observed no deaths from the infection, but concluded that it has a weakening effect on the host.

Further experimental work is needed to confirm these results and determine the physiological mechanisms responsible for them. Such work might better be done with *Trypanosoma lewisi*, which reaches much higher density in the circulating blood than *T. duttoni* and should have a correspondingly greater effect.

SUMMARY

Mice infected with the "non-pathogenic" *Trypanosoma duttoni* were subjected to stress in the form of partial starvation and low environmental temperature. Many of the starved mice died, while none of the controls did. The starved mice subjected to low temperatures died much earlier than did those kept at room tem-

perature. Parasitized mice which were starved died significantly earlier than non-parasitized mice. Parasitized mice which were not starved averaged only half as much weight gain as did non-parasitized mice. These findings suggest that *Trypanosoma duttoni* has a harmful effect on its host, under favorable conditions as well as under conditions of stress.

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RESEARCH NOTE

A POSSIBLE EFFECT OF TESTOSTERONE ON THE DEVELOPMENT OF *SCHISTOSOMA MANSONI*

An unusual ratio of sexes appeared in the offspring of *Schistosoma mansoni* treated with testosterone, there being 667 male worms, 434 females, and 240 male-like hermaphrodites in the group. The circumstances of the experiment, here described, suggest that the testosterone may have been the cause of the numerous hermaphrodites.

For the first generation of worms, pooled cercariae from snails infected with many miracidia were used to expose 20 mice. Ten of these mice each received 26 mg of crystalline testosterone in 23 injections over a period of 49 days. The rest of the mice received the same number of saline injections. On the 49th day, eggs were collected from 4 of the mice receiving testosterone, and snails were exposed to pooled miracidia which hatched from the eggs. Each snail was exposed to 15 miracidia, and most or all penetrated in each case.

Five mice later were exposed to pooled cercariae shed by 11 of these snails. One mouse died, and the worms recovered from the remaining 4 hosts had the sex ratio given above. The proportion of sexes and hermaphrodites was about the same in each individual mouse as in the total.

The sex ratio in worms taken from 34 mice not subjected to experimental procedures, after exposure to pooled cercariae from snails infected with many miracidia, was 1321 males, 1314 females, and 8 hermaphrodites. Worms from several experimental hosts, including the parent worms subjected to testosterone, had a similar ratio. No individual mouse had worms with an exceptional imbalance of sexes, nor harbored more than 2 hermaphrodites. Any imbalance usually was in the direction of an excess of females.

Spontaneous hermaphroditism in a substantial proportion of worms is not known to occur in *S. mansoni* developing in the laboratory mouse, although it has been reported when such animals as the guinea pig are used as the host. Spontaneous hermaphroditism in excess of 1% of the total number of worms has not been found in this laboratory in the strain of *S. mansoni* used, nor have larger percentages been reported in the literature. The appearance of so many hermaphrodites among the offspring of worms subjected to testosterone, and in no other group, makes it reasonable to believe that the testosterone was directly or indirectly responsible. The hermaphrodites and females together almost exactly equalled the number of males. This could be a coincidence, but it also could indicate that the hermaphrodites are reversed females. The experiment is being repeated, with modifications designed to elucidate answers to some of the problems posed.—EDWIN J. ROBINSON, JR., *Department of Biology, Kenyon College, Gambier, Ohio.* This investigation was supported by research grant E-975 from the National Institute of Allergy and Infectious Diseases, Public Health Service.

STUDIES ON THE GENUS *ACANTHATRIUM* FAUST, 1919 (TREMATODA: LECITHODENDRIIDAE); WITH THE DESCRIPTION OF TWO NEW SPECIES

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The genus *Acanthatrium* Faust, 1919, (Lecithodendriinae Luhe, 1901, Lecithodendriidae Odhner, 1910) was erected to include the type, *A. nycteridis* Faust, 1919. Faust considered the presence of spines in the genital atrium of sufficient diagnostic importance to erect the new genus. He transferred to this new genus, *Lecithodendrium sphaerula* Looss, 1896, designating it *Acanthatrium sphaerula*. Since that time several new species of this genus, which it limited so far to parasites of chiropterans, have been described. These include *A. eptesici* Alicata, 1932, *A. molossidis* Martin, 1934, *A. oregonense* Macy, 1939, *A. ovatum* Yamaguti, 1940, *A. alicatai* Macy, 1940, *A. microcanthum* Macy, 1940 and *A. pipistrelli* Macy, 1940. Recently Sogandares-Bernal (1956) described *A. jonesi* and *A. macyi* from Korean bats.

During a survey of bat parasites in several counties of Virginia and Pennsylvania, the author came across 2 new species which are described in this paper. These flukes were recovered from the small intestine of bats identified as *Eptesicus f. fuscus* (Beauvois) captured in Tazewell and Russell Counties, Virginia, January, 1956, while they were in hibernation. The trematodes were fixed in Carnoy's (6:1:1), stained with Harris' alum hematoxylin and cleared in xylene.

Macy (1940) mentioned, as a confusing species, *Lecithodendrium japonicum* Yamaguti, 1940. Skarbilovich (1948) transferred this species to the genus *Acanthatrium* and the subgenus *Mesothatrium*. Sogandares-Bernal (1956) raised this subgenus to the generic level. Having reviewed the original description, the author agrees with Skarbilovich that this trematode belongs in the genus *Acanthatrium* since the distinct atrial spines, characteristic of *Acanthatrium*, are present. Furthermore, the author proposes the complete elimination of the subgenus *Mesothatrium*. The internal organs of this Japanese species closely approximate those of the other members of the genus in measurements and positions. The principal difference lies in the fact that the vitellaria of *A. japonicum* lie posterior to the level of the testes instead of anterior as in the other species. Thus a revision of the original generic description by Faust (1919) and the once revised description by Alicata (1932) is made to read as follows:

Acanthatrium: Small flukes, spherical to pyriform, with a genital atrium lined and/or bordered with spines; prostate cells numerous; testes in actabular or pre-acetabular zones; vitellaria anterior or posterior to the testes or both; excretory system, according to Faust (1919), composed of 4 groups of flame cells for each half of the body, each group containing 3 flame cells. Parasites of intestines of bats. Type species; *A. nycteridis* Faust, 1919.

Acanthatrium amphidymum n. sp.

Ten specimens of this new species were recovered from the small intestine of the bat, *Eptesicus f. fuscus* from Tazewell County, Virginia. The specific name *amphidymum* is derived from the Greek word "amphidymus" meaning "chambered," used in this case since the genital

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atrium of the trematode is partially separated into 2 chambers. All measurements, except where indicated, represent the average, and are in millimeters.

Specific diagnosis: Aspinous body pyriform, 0.644 to 0.504 long and 0.56 to 0.44 wide. Oral sucker subterminal, 0.08 in greatest diameter. Short esophagus 0.023 in length, extending posteriad along mid-line to just above anterior border of prostate mass, here it divides into intestinal ceca which terminate at anterior margins of left and right testes. Acetabulum in center of body slightly overlapping prostate mass, 0.075 in diameter. Oval testes at same level as posterior half of prostate mass. Left testis 0.129 by 0.141; right testis, 0.110 by 0.139. An efferent duct arising from medio-lateral border of each testis; each duct joining anterio-medially to form common vas deferens which enters an inconspicuous seminal vesicle that connects anteriorly to an enlarged ejaculatory duct lying in left median plane of prostate mass, near genital pore. Pyriform ovary, 0.151 by 0.076, posterior to right testis and partially covered by it. Small oötype lies mesial to posterior tip of ovary. Oötype opens into uterus on dorso-medial surface. Uterine coils, filled with eggs, occupy most of space between posterior body wall and posterior margins of testes and acetabulum. Uterus eventually directed cephalad and opens into genital pore dorsal to prostate mass. Prostate mass 0.178 by 0.148, made up of glandular cells, covered by transparent membrane, occupying area between 2 testes. Genital atrium, in middle of prostate mass, partially divided into 2 chambers by horizontal glandular wall, arising from right inner surface of atrium. Both chambers of atrium lined with 1 row of lanceolate spines with mean length of 14 microns. Spines of anterior chamber completely line atrial wall and pointed posteriad; less numerous spines of posterior chamber pointed toward left anterio-lateral corner. Genital pore in mid-ventral wall of genital atrium. Vitellaria composed of large follicles, limited to 2 areas anterior to intestinal ceca. From posterior margins of vitellaria arise respective vitelline ducts which proceed posteriad to just below acetabulum where they join to form common duct which enters oötype. Eggs 0.026×0.016.

Host: Eptesicus f. fuscus (Beauvois)
Habitat: Small intestine.
Locality: Tazewell County, Va.
Type specimen: U. S. Nat. Mus. Helminth. Coll. No. 38173.
Paratypes in author's collection.

Acanthatrium oligacanthum n. sp.

Five specimens of this species were recovered from the small intestine of 2 bats, *Eptesicus f. fuscus* from Russell County, Va. The specific name *oligacanthum* is from the Greek words "oligos" meaning "few" and "acanthum" meaning "spine." In this case reference is made to the few small atrial spines which are characteristic of this new species. All measurements, except where indicated, represent the average, in millimeters.

Specific diagnosis: Spinous body pyriform to oval, 0.49 to 0.38 long, 0.322 to 0.238 wide. Subterminal oral sucker 0.081 in diameter. Prepharynx absent. Muscular pharynx 0.037 in diameter. Short esophagus, 0.018 in length, bifurcates into intestinal ceca in region just anterior to prostate mass. Ceca of typical lecithodendriid type. Acetabulum 0.055 in diameter, situated between testes. Testes subspherical, in mid-region of body; right testis 0.107 by 0.098, left testis 0.108 by 0.09. Two vasa efferentia rise from respective anterio-lateral margins of testes and unite anterio-medially, just below the prostate mass, as common vas deferens. Anteriorly vas deferens enters indistinct seminal vesicle which leads into thicker ejaculatory duct. Ovary pyriform, 0.088 by 0.065, situated posterior to right testis and partially covered by it. Ovary gives rise posteriorly to short oviduct which proceeds to area just below acetabulum where it enters oötype. Uterus, filled with yellowish-brown eggs, 0.027 by 0.019, occupies most of area posterior to lower margins of testes and acetabulum. Medium-sized vitelline follicles confined to each side of esophagus and in front of testes; from posterio-medial margins of 2 vitelline masses, arise respective vitelline ducts which unite posteriorly just anterior to oötype and enter as a common duct. Prostate mass, 0.071 by 0.075, occupies region bordered by angle of intestinal ceca. Genital atrium in right upper quadrant of prostate mass, lined along 1 wall with 9 minute spines, 0.002 long, pointed dorso-medially. Genital pore in middle of prostate mass.

Host: Eptesicus f. fuscus (Beauvois)
Habitat: Small intestine.
Locality: Russell County, Va.
Type specimen: U. S. Nat. Mus. Helminth. Coll. No. 38174.
Paratypes in author's collection.

DISCUSSION

The accompanying table indicates that the dimensions of the body and the internal organs are not good criteria for separating the species of *Acanthatrium*

since several species are of comparable sizes. The main differentiating criterion appears to be the one most authors have used namely the position and arrangement of the atrial spines.

A. amphidymum n. sp. closely resembles *A. nycteridis Faust,* 1919 and *A. alicatai* Macy, 1940. However, it can be separated from these 2 species since the atrial spines of the new species are arranged in 2 horizontal chambers of the genital atrium rather than in 3 opposing diverticula as found in *A. nycteridis* and *A. alicatai.*

A. oligacanthum n. sp. bears the closest resemblance to *A. microcanthum* and *A. molossidis;* however, the many atrial spines of *A. microcanthum* (0.003–0.007) are distinctly arranged in a circumferential pattern, while those of *A. molossidis* (0.005) are arranged in no definite pattern but are more in number and larger in size than those of *A. oligacanthum.* Furthermore, the absence of cuticular spines again separates *A. oligacanthum* from *A. molossidis.*

Because of the revision of the diagnostic characteristics of *Acanthatrium* and the general acceptance of the genera *Prosthodendrium* Dollfus, 1931 and *Paralecithodendrium* Odhner, 1911, in addition to *Lecithodendrium* Looss, 1899, the following key to the genera of subfamily Lecithodendriinae occurring in mammals is proposed.

Key to the Genera of Lecithodendriinae Occurring in Mammals.

1. Cirrus pouch absent
   - A. Cuticle completely spinous; testes enveloped in the folds of the uterus *Pycnoporus* Looss, 1899.
   - B. Cuticle smooth or only partially spinous
     - a. Genital atrium armed with spines; vitellaria anterior and/or posterior to testes *Acanthatrium* Faust, 1919.
     - b. Genital atrium not armed with spines
       - i. Vitellaria situated in front of testes; ovary not lobed *Prosthodendrium* Dollfus, 1931.
       - ii. Vitellaria situated posterior to testes ..... *Lecithodendrium* Looss, 1899.
       - iii. Ovary lobed and usually anterior to the acetabulum; vitellaria anterior to testes *Paralecithodendrium* Odhner, 1911.
2. Cirrus pouch present
   - A. Genital pore in vicinity of pharynx ..... *Phaneropsolus* Looss, 1899.
   - B. Genital pore near the acetabulum ..... *Parabascus* Looss, 1907.

Macy (1940) gave a key to the genus *Acanthatrium.* However he failed to include *A. eptesici.* With the addition of the 2 species from Korean bats described by Sogandares-Bernal and the 2 new species described in this paper, it is felt that the following key to the species will be useful.

Key to the genus *Acanthatrium* Faust, 1919

1. Esophagus absent
   - A. Cuticle spinous in anterior one-third of body ..... *A. molossidis* Martin, 1934.
   - B. Cuticle aspinous; atrial spines 10–15 microns long, arranged as a crescent *A. oregonense* Macy, 1939.
2. Esophagus present
   - A. Genital atrium partitioned
     - a. Atrium partially partitioned into two chambers ..... *A. amphidymum* n. sp.
     - b. Atrium completely partitioned into two vertical chambers, only one lined with spines; vitellaria post-testicular ..... *A. japonicum* (Yamaguti, 1939), Skarbilovich, 1948.
     - c. Atrium partially partitioned into three lobes
       - i. Atrial spines 10–15 microns long ..... *A. nycteridis* Faust, 1919.
       - ii. Atrial spines few, larger, 22–26 microns ..... *A. alicatai* Macy, 1940.
   - B. Genital atrium not divided
     - a. Atrium with a conical diverticulum lined with spines ... *A. ovatum* Yamaguti, 1939.

b. With parallel atrial spines pointed posteriad, 25 microns long
A. eptesici Alicata, 1932.

c. With one group of atrial spines pointed posteriad and two groups on each side pointed mediad, 25 microns long ..... *A. pipistrelli* Macy, 1940.

d. With innumerable atrial spines, circumferentially arranged, 3–7 microns long
A. microcanthum Macy, 1940.

e. With nine minute atrial spines, 2 microns long, in a row .... *A. oligacanthum* n. sp.

f. With blunt spines located just anteriad of utero-atrial junction, 7–12 microns long
A. macyi Sogandares-Bernal, 1956.

C. With a multi-lobed ovary; atrium completely lined with spines
A. sphaerula (Looss, 1896), Faust, 1919.

D. With a lobed acetabulum; numerous minute atrial spines at anteriormost end of genital atrium ..... *A. jonesi* Sogandares-Bernal, 1956.

Table of Comparative Measurements

	A. sphaerula	A. nycterides	A. eptesici	A. molossidis	A. oregonense	A. ovatum	A. alicatai	A. microcanthum	A. pipistrelli	A. amphidymum	A. oligacanthum	A. japonicum
Body length	1.32	0.185 -0.200	0702 -1.200	0.920	0.650 -0.820	0.625 -0750	0.650 -0.810	0.900 -1.100	0.600	0.573	0.442	0.420 -0.600
Body width	1.40	0.150 -0.160	0.468 -0764	0.870	0.650 -0.850	0.425 -0.470	0.650 -0.890	0.650 -0700	0.480	0.528	0.291	0.250 -0.400
Cuticula	Asp	Asp	Asp	⅓ sp	Asp	Asp	Asp	Asp	Asp	Asp	Asp.	Asp
Oral sucker	0.18	0.025	0.106 x 0.106	0.100	0.078 x 0.087	0.094 x 0.108	0.090 x 0.100	0.140 x 0.110	0.110	0.080	0.081	0.054 -0.057
Acetabulum	0.18	< 0.16	0.080 -0.095		0.085 -0.090	0.075 -0.090	0.075 -0.095	0.120 -0.150	0.120 -0.130	0.075	0.055	0.040 -0.060
Pharynx		0.006 x 0.005	0.041 -0.053	0.050	0.031 -0.045	0.360 -0.050	0.040 -0.050	0.040 -0.060	0.026 -0.040	0.044	0.037	0.021 x 0.030
Esophagus		0.008	0.034 -0.076	none	none	0.090	moderate length	long	extremely short	0.023	0.018	0.030 -0.040
Testis	0.19 - 0.28	0.037 x 0.028	0.189 x 0.148	0.140 - 0.160	0.140 - 0.180	0.134 x 0.117	0.123 x 0.190	0.190	0.100 - 0.120	0.140 x 0.119	0.107 x 0.094	0.076 x 0.069
Prostate mass		0.058	0.224 x 0.274	0.280 x 0.220	0.160		0.210	0.170	0.140	0.178 x 0.148	0.071 x 0.075	
Spines		10-15u	25u	5u	10-15u	12u 18u	22-26u	3-7u	25u	14u	2.6u	6u
Ovary	multi-lobed	0.230 x 0.340		0.200 x 0.120	0.090 x 0.160	0.460 x 0.450	0.150 x 0.120	0.150 x 0.150	0.070 x 0.050	0.151 x 0.076	0.088 x 0.065	0.049 x 0.060
Egg	19u x 10u	44u x 23u	30u x 15u	30u x 20u	30u x 16u	33u x 18u	32u x 18u	27u x 14u	24u x 13u	26u x 16u	27u x 19u	31u x 18u

Bhalerao (1926) described a trematode from the bat, *Nyctinomus plicatus*, in Burma. He believed this fluke to be morphologically identical with *A. nycteridis*. However, the uterine coils were arranged transversely, and the measurements of the body and both suckers were larger than those reported by Faust. He proposed a new variety, *A. nycteridis plicati*. Hence the genus now includes 14 species and one variety.

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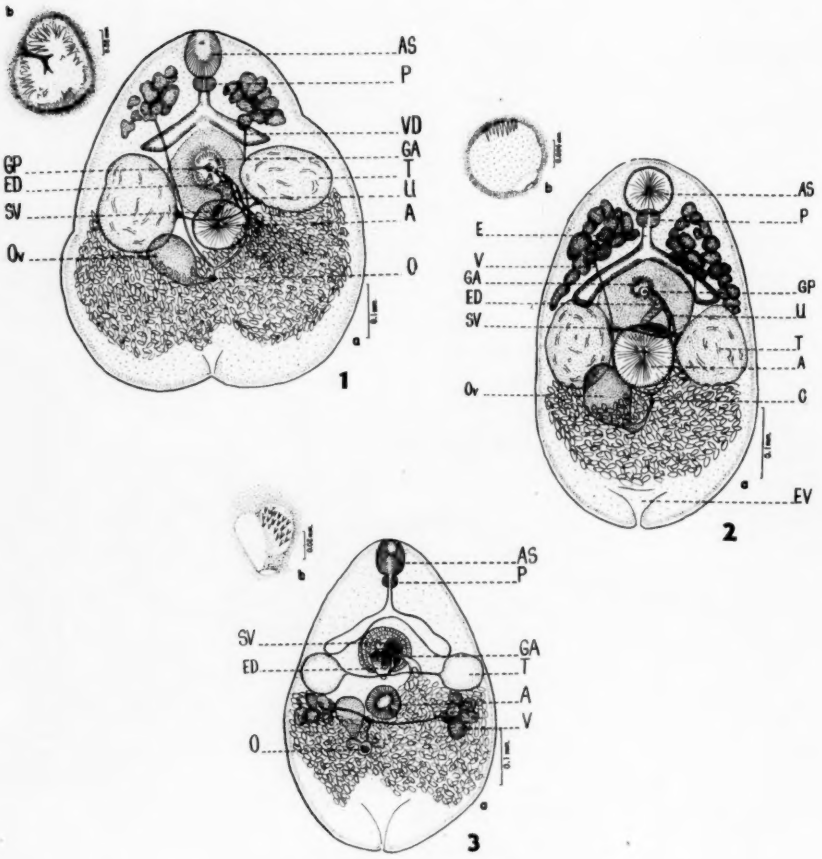

EXPLANATION OF FIGURES

FIGURE 1. a. *Acanthatrium amphidymum* n. sp. Ventral view. Camera lucida drawing.

A. Acetablum.	GP. Genital pore.	SV. Seminal vesicle.
AS. Anterior sucker.	O. Oötype.	T. Testis.
ED. Ejaculatory duct.	Ov. Ovary	U. Uterus.
GA. Genital atrium.	P. Pharynx.	VD. Vitelline duct.

b. Enlarged camera lucida drawing of genital atrium showing the arrangement of the atrial spines.

FIGURE 2. a. *Acanthatrium oligacanthum* n. sp. Ventral view. Camera lucida drawing.

A. Acetabulum.	GA. Genital atrium.	SV. Seminal vesicle.
AS. Anterior sucker.	GP. Genital pore.	T. Testis.
E. Esophagus.	O. Oötype.	V. Vitellaria.
ED. Ejaculatory duct.	Ov. Ovary	U. Uterus.
EV. Excretory vesicle.	P. Pharynx.	

b. Enlarged drawing of the genital atrium showing the arrangement of the atrial spines.

FIGURE 3. a. *Acanthatrium japonicum* (Yamaguti, 1939). Ventral view.

A. Acetabulum.	GA. Genital atrium.	SV. Seminal vesicle.
AS. Anterior sucker.	O. Oötype.	T. Testis.
ED. Ejaculatory duct.	P. Pharynx.	V. Vitellaria.

b. Enlarged drawing of the genital atrium showing the position and arrangement of the atrial spines.

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RESEARCH NOTE

STOMACH INFECTIONS WITH *TRYPANOSOMA CRUZI*

During the course of research work with *Trypanosoma cruzi* the authors autopsied 30 Swiss Albino (CFw) mice, which had been experimentally infected with *T. cruzi* (from culture or from infected mice). The mice died or were sacrificed at intervals ranging from 2 weeks to 3 months. In some of the animals trypanosomes were never observed in direct or stained blood smears. However, in all cases necrosis of the upper third of the stomach was noted (Fig. 1). Histological sections of the diseased tissue stained with hematoxylin-eosin or Giemsa

FIGURE 1

FIGURE 2

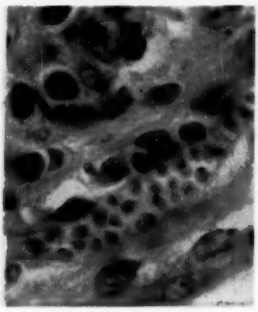

FIGURE 3

revealed the presence of leishmania in the serosa (Fig. 2) and the muscle layers (Fig. 3). Leishmania forms were also found in the muscles of the heart, esophagus, and small intestine.

Porto (Rev. Goiana de Med. **1:** 43–54, 1955—from Trop. Dis. Bul. **53:** 1105, 1956) suggested that the stomach is involved in Chagas' disease. The above observations in mice lend credence to this hypothesis, and it is recommended that in endemic areas *T. cruzi* be considered as a possible etiological agent for otherwise unexplained gastric disturbances. Further studies, however, would be required to determine whether stomach involvment is restricted to certain strains of *T. cruzi*, or whether it occurs in all infections with this parasite.

The authors wish to thank Dr. J. F. Pilcher (Corpus Christi) for supplying the strain of *T. cruzi* used in these studies; it was obtained from the first reported indigenous human case of Chagas' disease in the United States. We also thank the Brooke Army Hospital and the Fourth Army Central Photo Laboratories for the photographs.—ELLIOTT LESSER, 1st Lt., MSC, and JOHN M. LUKEMAN, Lt. Col., M. C., *4th Army Area Medical Laboratory, Fort Sam Houston, Texas.*

THE LIFE CYCLE OF *SKRJABINOPTERA PHRYNOSOMA* (ORTLEPP) SCHULZ, 1927 (NEMATODA: SPIRUROIDEA), A GASTRIC NEMATODE OF TEXAS HORNED TOADS, *PHRYNOSOMA CORNUTUM*.[1]

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The author previously (Lee, 1955) reported that the female of *Skrjabinoptera phrynosoma* must be expelled by its definite host and that its body wall serves as a protective covering for the larvae within the eggs which are enclosed by five-layered capsules. This mode of egg dispersal, which seems to circumscribe the availability of the egg to its prospective intermediate hosts, is apparently not to the interest of the worm. However, the possibility occurred to the author that there might be some unique adaptations of the life cycle of this worm.

MATERIALS AND METHODS

Horned toads were kept in large cardboard boxes and were fed with Texas agricultural ants, *Pogonomyrmex barbatus* var. *molefaciens* Buckley, 1866, every other day. Ground beetles and other arthropods were used when available. Dry female worms were collected as previously described (Lee, 1955).

Horned toads under 1 year old were kept in an outdoor pen, 4 feet long and 4 feet wide, constructed of a strip of tin 16 feet long and 18 inches wide. Eight inches were buried under the ground and 10 inches were exposed. The site of the pen was so chosen that there would be enough vegetation for shade and enough open space for runways. Salted meat, boiled raisins, and molasses were placed inside the pen from time to time to attract smaller ants, such as *Forelius foetida* (Buckley) and *Pseudomyrma brunnea* F. Smith. The tin strip differentially excluded the agricultural ants which were unable to climb the smooth surface of the tin strip.

In experimental feeding, horned toads were marked on the abdomen with India ink. Molting horned toads were isolated and remarked after the molting process was complete.

Agricultural ants were collected with a pair of forceps or by traps, consisting of 4-inch-tall drinking glasses buried strategically around the ant nest, with the rim of the glass level to the surface of the ground. In feeding horned toads, ants were introduced about 10 at a time to avoid stinging, which could cause temporary frenzy or death.

Texas agricultural ants used in attempts to infect the intermediate host were kept in Stansi ant nests, Standard Science Supply Co., 1231–39 N. Honore St., Chicago 22, Ill. Battery jars half filled with sterilized soil were also used for housing the ants but were found unsatisfactory for experimental feeding.

Other arthropods used in experimental feeding were raised in the laboratory.

Both adult and larval *S. phrynosoma* were fixed in hot 70% alcohol, cleared in lacto-phenol, and preserved in 70% alcohol with 3% glycerine.

Cysts used for feeding experiments were dissected freshly from the infected

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[1] Adapted from a portion of Ph.D. thesis submitted to the Graduate School of the University of Minnesota.

ants into 0.8 per cent saline solution and were introduced to the horned toads by means of medicine dropper.

OBSERVATIONS

Experimental Feeding of Certain Coleoptera and Orthoptera

Eggs from freshly expelled worm were given to Texas agricultural ants, German cockroaches, *Tribolium* beetles, and varied carpet beetles, *Anthrenus verbasci* (Linn.). All these were opened within two weeks after feeding and were found negative for nematode larvae.

Dry female worms or egg capsules were fed to 18 German cockroaches, 20 *Tribolium* beetles, 20 Texas agricultural ants, and 20 larvae of varied carpet beetle, all with negative results. The larvae of the varied carpet beetle consumed the dry worm readily, but always left the uterine side intact. The roaches and *Tribolium* beetles did not feed on the dry worms.

Egg capsules containing second stage larvae were given to 16 German cockroaches, 20 larvae of *Tribolium,* and 20 larvae of varied carpet beetle. All of these arthropods were found negative for nematode larvae upon dissection three days after feeding.

Discovery of the Intermediate Host

One horned toad, which had been kept in the laboratory for 16 days, was dissected 48 hours after feeding. It was noticed that within the stomach one ant appeared to be biting on an immature worm, but closer examination revealed that the posterior end of the worm extended from the ant's head through a break in the side of the mandible. Two more immature worms were emerging from the anterior end of the gaster. All worms were alive when found. The one found in the head of the ant remained attached to the mandible. The two worms found in the gaster were loosened through handling. One measured 8.2 mm long $\times$ 0.224 mm wide; one measured 6.1 mm long and 0.198 mm wide. The dentition and the number and arrangement of the cephalic papillae were identical with those of *S. phrynosoma.* Their esophagi were also similar to that of *S. phrynosoma,* viz., a bulbless structure divided into anterior muscular and posterior glandular parts. The digestive and excretory systems of both worms were well developed. The genital primordia, club-shaped, were found at the junction of the anterior and middle thirds of the body. The larger (8.2 mm) worm's genital primordium measured 0.149 mm $\times$ 0.034 mm. The smaller (6.1 mm) worm's genital primordium measured 0.116 mm $\times$ 0.02 mm. Thus the two larvae from the gaster were suspected of being the larval stages of *S. phrynosoma.*

This discovery resulted in intensified efforts to infect Texas agricultural ants. (Hereafter, the words "ant" or "ants" will refer to Texas agricultural ant, *Pogonomyrmex barbatus* var. *molefaciens* unless otherwise stated). One dry worm kept two years in the laboratory at room temperature was given to 25 ants in one Stansi ant nest. These ants had been starved for 3 days prior to the introduction of the dry worm to the nest. Ten days later 16 ants were dissected. One of these 16 ants yielded 11 immature nematode larvae from its gaster. These larvae were coiled in separate thin membraneous cysts loose in the body cavity of the ant. Some cysts contained 2 or 3 larvae which were identical with the 3 emerging larvae described above. The remaining 9 ants were dissected on the twelfth day, and

one of these contained 9 larvae in its gaster. All the larvae were at about the same stage of development.

The larvae found within these 2 ants appeared too advanced in their development to be the result of the experimental feeding, as it seemed very unlikely that the larvae of *S. phrynosoma* could develop from 0.4 mm to 6 mm within 12 days. Therefore the author collected 11 ants from the same nest the next day and upon dissection 3 of these 11 were found to harbor larval nematodes. The number

TABLE I — *Summary of experimental feeding of horned toads 1 to 27, x-1 to x-30*

Horned Toad No.	Horned Toad Size (snout-vent in mm.)*	No. of Larvae Given	No. of Worms Recovered	Duration of Experiment (in days)
1	40	9	7	3
2	32	10	7	5
3	33	10	10	26
4	33	2	2	19
5	38	9	9	65
6	45	10	10	46
7	25	3	3	14
8	24	3	3	14
9	24	2	2	14
10	23	2	2	14
11	22	4	4	14
12	25	4	4	14
13	23	4	4	19
14	23	3	3	19
15	23	4	4	19
16	23	5	5	19
17	24	5	3	19
18	23	6	6	19
19	23	5	5	19
20	24	4	4	19
21	25	4	3	23
22	25	4	2	23
23	22	4	4	23
24	22	4	3	23
25	23	4	4	23
26	22	4	2	34
27	25	3	3	14
x-1 to x-30	22-50	0	0	6-65

* Measured at the end of experiment.

of larvae in each gaster was 6, 9, and 18. Thus it was highly probable that the two previous ants were naturally infected. Subsequent finding revealed the number of larvae in each ant to be 1 to 75.

It was observed that the gasters of infected ants were distended and lighter in color, particularly those with more than 10 larvae. However, gasters of ants that harbored 1 or 2 larvae might appear normal. Dissection of 76 ants with distended gaster showed 16 ants, or 21%, to be positive for nematode larvae. It was evident

that heavy infection with nematode larvae caused distension and lightness of the color of the gaster, though other unknown factors could produce the same appearance. Selection of ants with light-colored and distended gaster invariably yielded higher infection rate.

Infecting Horned Toads with Larvae from Intermediate Host.

Since a majority of the adult horned toads harbor *S. phrynosoma* and because no effective methods were found of ridding the horned toads of worms, only young horned toads were used for experimental feeding. After dissecting over 300 horned toads of various size it was observed that horned toads under 50 mm from snout to vent never had any agricultural ants or worms in their stomachs. The food of these young horned toads consisted mainly of smaller ants, *Crematogaster* sp., *Forelius foetida* (Buckley), and *Pseudomyrma brunnea* F. Smith.

Twenty-seven horned toads marked 1, 2, 3, etc. which had been given cysts were placed in the outdoor pen with 30 controls marked x-1, x-2, x-3, etc. Both groups were composed of horned toads measuring between 22 and 50 mm from snout to vent.

The experimentally infected horned toads were dissected at various intervals as indicated in Table I. Male worms with distinct *S. phrynosoma* type bursae were recovered in horned toads No. 4, 5, 18, 19, 21, and 25. At least 19 days were required for the completion of the male reproductive system, including the bursa. Eggs were found in females which were 65 days old. All 27 toads were positive and all except 5 yielded the exact number of worms that they were fed.

The 30 controls were also dissected at various intervals, from 6 to 65 days. All were negative. It is therefore concluded that the *S. phrynosoma* found in horned toads 1 through 27 were the result of experimental feeding and not of natural infection.

Morphology of Infective Larvae

Fifty fixed larvae dissected from the gaster of adult worker ants were measured. Results are summarized in Table II. The average length of the larvae was 5.93

TABLE II — *Distribution of the length of 50 larvae, randomly chosen, found in the gasters of the adult workers of Texas agricultural ants*

Length Range (in mm.)	Per cent
3.5 - 3.9	2
4.0 - 4.4	0
4.5 - 4.9	2
5.0 - 5.4	24
5.5 - 5.9	8
6.0 - 6.4	42
6.5 - 6.9	6
7.0 - 7.4	10
7.5 - 7.9	6

mm. Although the shortest one measured only 3.5 mm, its digestive and excretory systems appeared to be as well developed as those of any of the others.

General details of the digestive and excretory systems of the infective larva are described below. All the measurements were taken from 1 larva, 6 mm long and 0.370 mm at the widest portion of its body, which fell within the modal length of those measured and was very close to the average size.

There is a distinct slender stoma or pharynx, 0.032 mm long, devoid of any teeth or stylets. Posterior to the pharynx is the muscular portion of the esophagus, 0.957 mm long and 0.092 mm wide. The syncytial glandular portion is slightly wider, 0.096 mm, and measured 1.254 mm long. Total length of the esophagus is 2.211 mm. The entire esophagus is lined with wavy cuticle. A distinct esophago-intestinal valve separates the esophagus and the intestine.

Immediately posterior to the esophageal valve the intestine is 0.120 mm wide. The rest of the intestine has the same width, except that it is constricted abruptly to form the rectum. The rectum is 0.128 mm long, its base 0.112 mm wide, and its apex, the anus, 0.036 mm wide. The distance between the anus and the caudal tip of the infective larva is 0.165 mm. Externally, the intestine is covered with polygonal cells. Within its lumen there are some yellowish granules. Since the infective larva is enclosed in a thin cyst, the source of this yellowish granulated matter is unknown. When the infective larva was released from the cyst, many of these yellowish particles were expelled through the anus. This yellow matter was also observed in some of the unbroken cysts.

The excretory system of the infective larva is of the H type, with 2 ventral glands, each measuring 0.048×0.027 mm. There is a large nucleus, 0.012 mm in diameter, within each gland, and each nucleus had a distinct nucleolus. The excretory pore is situated ventrally, flush with the junction of the muscular and glandular esophagus. Excretory pores situated slightly posterior to the junction were observed in other infective larvae. The terminal duct is about 0.032 mm long and 0.001 mm wide. It widens slightly as it joins with the ventral glands and the arcade. The two lateral ducts are spindle-shaped, with thick syncytial walls. The nuclei of the wall are widely scattered. The widest portion of the lateral wall is posterior to the esophageal valve. It measures about 0.104 mm in width; then it tapers off gradually toward both ends. The portion of the lateral duct anterior to the arcade measures about 0.096 mm in length.

Among the fifty infective larvae studied for Table II, 9 were male, all of which possessed an incipient male reproductive system. One of the 9 was used for measurement. This particular larva was 6.1 mm long and 0.356 mm wide. Its reproductive system consisted of a testis, 0.024×0.032 mm, and a tubule tapering off posteriad from the testis. The testis was situated 2.864 mm behind the cephalic end.

The female reproductive system in the 41 female larvae consisted of a germ cell mass in the form of a club which measured about 0.159 mm long and 0.03 mm at its thickest end.

Infection in the Intermediate Host

After repeated negative results in feeding egg capsules to workers of Texas agricultural ants, the author began to suspect that there was an age resistance among the ants. In order to test this hypothesis, the author dug 4 ant nests from which infected ants had been taken. Two of these nests yielded many larval ants in their last stadium, pupae, and newly-emerged ants, the callows, characterized

by their white color. Upon dissection, 3 of 68 callows, 4 of 53 pupae and 3 of 70 larvae were found to harbor cysts of *S. phrynosoma*. The youngest cyst was found in the larval ant. The cyst measured about 0.633 mm in diameter and contained many fat droplets. The larva within the cyst measured 0.541 mm long and 0.044 mm wide at its widest portion. This larva, morphologically, was intermediate between the second stage larvae found in the egg capsule and those found in the adult worker ants. The body of the second stage larva was divided roughly into three distinct sections, the esophageal, the intestinal, and the caudal. The esophageal section barely showed the rudiment of the esophagus. The intestinal rudiment in the second body section was very distinct; there was a short light space about 0.033 mm long immediately behind the junction between the esophagus and the intestine; the rest of the intestinal rudiment was opaque. There were about 6 cells tandem posteriad to the opaque portion of the intestine. The body of the larva found in the youngest cyst within the gaster of larval ant was also divided distinctly into 3 sections. The intestinal rudiment also had a light space about 0.011 mm long, immediately behind the junction between the esophagus and the intestine. However, the opaque portion of the intestine contained many fat droplets. The esophagus of the larva within the youngest cyst was well developed. The entire lumen of the esophagus was now lined with cuticle. There was a distinct esophageal bulb. Immediately behind the intestine the 6 tandem cells were replaced by a columnar structure which connected the intestine and the site of future anal opening. The rudiment of the excretory system was now developed into an oval mass, 0.064 mm long, 0.016 mm wide at its widest portion, without any tubes or opening. The rudiment of the genital organ remained invisible at this stage. The tail became abruptly pointed as compared with the gradual tapering off in the second stage larvae. There was a distinct stylet at the cephalic end.

Cysts found in the pupae of the ants varied in size according to the age of the pupa. The cysts found in young pupae were about the same size as those found in the larval ants. However, the worm inside the pupa was much larger, about 1.114 mm in length. Fat droplets were less abundant as compared with cysts found in the larval ants. The digestive system became a hollow tube with thin membranous walls.

In still older cysts as found in the callows, the fat droplets had almost totally disappeared. A homogenous brownish substance now occupied the cavity of the cyst. Later this brownish substance would disappear and one could observe 3 to 4 sac-like structures within the cyst's cavity. These sac-like structures inside the cavity of the cyst may be the molted skins of the larva of *S. phrynosoma*. In cysts found in the adult worker ants there were no sac-like structures.

The larvae of *S. phrynosoma* recovered from the callows of the ants averaged about 4 mm in length and 0.099 mm wide, whereas the average length of the larvae recovered from the adult worker ants was 5.93 mm and the width was 0.31 mm. The wall of the digestive system became thicker and food appeared in its lumen. The excretory system had all its tubules. The aperture of the excretory system protruded in the form of a papilla. The one papilla measured had a 0.02 mm base and 0.009 mm height. Both digestive system and excretory system were very slender, and thus the genital primordium was easily discernible. It appeared as a short curving rod, 0.064 mm long and 0.024 mm wide. The genital primordium

in larvae recovered from adult worker ants was obscured by the well-developed excretory systems.

From the study of the morphology of the various stages of the cysts found in the larvae, pupae, and adult ants, the author was led to believe that the cysts themselves originated from the ants. This belief is based on the following facts: (1) Cysts of other physalopterine worms studied are produced by the intermediate host (Schell, 1952). (2) The cysts found in the larval ants are similar in morphology to fat body trophocytes of the larval ant except for the presence of the larva. (3) The location of the cysts is the same as that where fat body trophocytes are found. It is believed that the second stage larvae of *S. phrynosoma* penetrates through the intestinal wall of the ant larvae and bores into one of the fat body trophocytes, abundant within the coelom of the larval ant, and lives on the contents of the fat body trophocyte. This explains the gradual disappearance of fat droplets within the cyst.

When the fat droplets inside the fat body trophocyte are totally consumed, the larva may live on the molted skins. This explains the absence or reduction in number of sac-like structures in older cysts. What the larva would live on after the consumption of the total contents of the fat body trophocyst and before being eaten by the horned toad poses an interesting problem. The author has casually observed that a few of the older larvae exist in the ant's gaster without a cyst. It is therefore possible that once the larva has consumed all the fatty substances and the molted skins within the fat body trophocyte, it may break through the cyst wall and live on other fat body trophocytes.

Callows, being negatively phototropic, will not emerge from their nest until fully mature and dark brown in color. Therefore, once the larvae of *S. phrynosoma* have gained access to the larvae of the ants, they will be sure to finish their development before being eaten by their definitive host.

The discovery of cysts within the larval ant plus repeated failure to infect adult ants indicates that the ants have an age resistance. The ants acquire the infection of *S. phrynosoma* during their larval stage. Since the fat body trophocytes are present both in the larvae and adult ants and because food habits are similar, there are few obvious physiological differences between these two stages of the ants. Possibly the age resistance is due to the morphological differences between the intestinal wall of the larval ant and that of the adult ant. It was observed that the intestinal wall of the larval ant was very thin as compared with that of the adult.

Attempts to Infect Other Vertebrate Hosts

Five salamanders, each of which had received 10 cysts of *S. phrynosoma,* were sacrificed after 9 days. All were found negative of any worms in their stomach or intestine.

Ten cysts and 5 adult worker ants with swollen gasters were given to 2 week-old chickens. A week later both were found negative for infection.

Five *Rana pipiens* Schreber received 10 cysts each. Four days later, upon dissection, all were found negative.

One collared lizard, *Crotophytus* sp., which received 10 cysts was dissected after 54 days; in its stomach one nematode was found which measured 6 mm in length. The dentition and the digestive tract appeared to be similar to that of

S. phrynosoma. The reproductive system was still in its rudimentary stage. Imbedded in the stomach wall of the lizard were 7 nematode cysts. One cyst contained 5 larval nematodes of various sizes which differed from the larvae of *S. phrynosoma* in many ways. The author did not attempt to identify them.

DISCUSSION

The intermediate host of *S. phrynosoma* has been proved to be the Texas agricultural ant, *Pogonomyrmex barbatus* var. *molefaciens.* The finding of S. *phrynosoma* infection in horned toads and other lizards where Texas agricultural ants are not found but where other species of the genus *Pogonomyrmex* are present, indicates that *S. phrynosoma* does not depend solely on the *barbatus* complex to complete its life cycle. Other genera of ants are not likely to be utilized by *S. phrynosoma* for intermediate hosts as indicated by the fact that young horned toads which live on smaller species of ants are free from *S. phrynosoma.*

The mode of egg dispersal and the morphological characteristics of the egg capsule of *S. phrynosoma* are unquestionably best adapted for an arid environment. However, these characteristics reduce the chance of contact with the intermediate host, as all the eggs are within the female body instead of being scattered throughout a wide area. Yet, this is compensated for by utilizing an ant for intermediate host. As the ant carries the expelled female to its nest, the eggs of the female worm thus become available to the entire ant population of the nest.

The infection rate among ants was found to be low, possibly due to faults of sampling. Even so, the low infection rate can still be compensated for by the astronomical number of ants consumed each day by each horned toad. One should not overlook the abundance of Texas agricultural ants within the natural niches of the Texas horned toads.

Once the author placed one dry female *Skrjabinoptera phrynosoma* in the middle of an ant runway to form a road block for some returning ants. One ant, after a moment of hesitation, abandoned the grass seed which was in its mouth, picked up the dry worm, and carried it to its nest. Further experimental studies by the author on the nutrition of Texas agricultural ants revealed that animal matter is indispensable to their well being.

SUMMARY

The intermediate host of *Skrjabinoptera phrynosoma,* a common stomach nematode of Texas horned toad, *Phrynosoma cornutum,* was proved to be the Texas agricultural ant, *Pogonomyrmex barbatus* var. *molefaciens.* The worm can infect only the larval stage of the ant.

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PLATE I

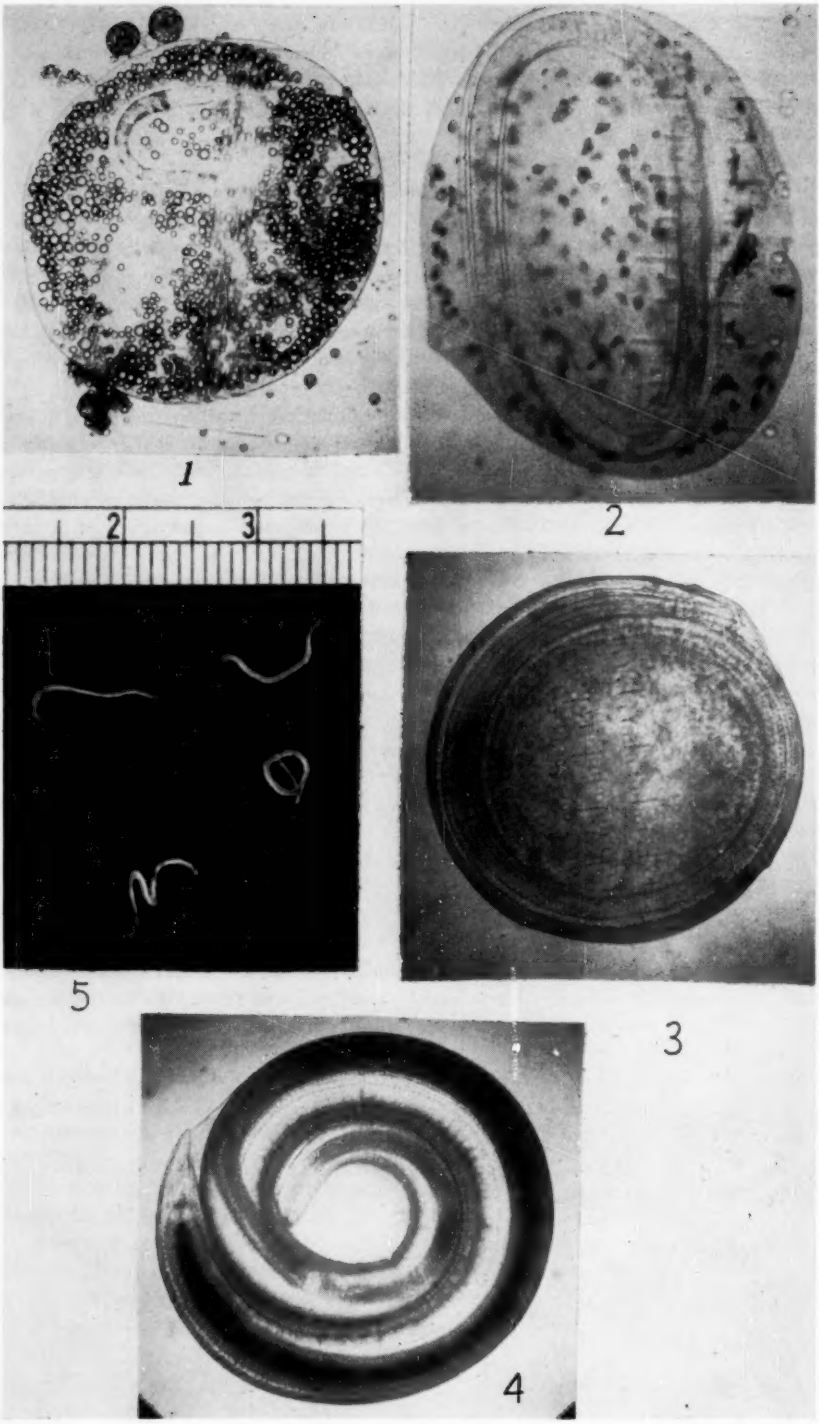
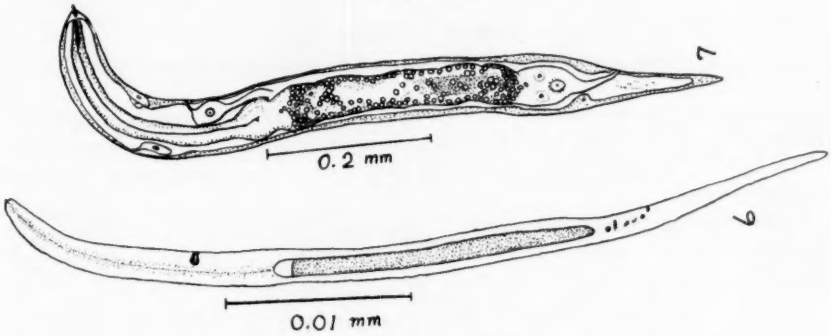


PLATE II



EXPLANATION OF FIGURES

PLATE I

FIG. 1. Cyst found in larval ant. Note the abundance of fat droplets and the relatively small size of the larva in comparison with the entire cyst. Each smallest division equals 0.0066 mm.

FIG. 2. Cyst found in the pupa of ant. Note the reduction of fat droplets and the increase in size of the larva.

FIG. 3. Cyst found in callow. Note the homogenous content within the cyst and the tremendous increase in size of the larva.

FIG. 4. Cyst found in the gaster of adult ant. Note the absence of substance within the cyst and the presence of substance within the digestive tract of the larva.

FIG. 5. Dry females. Note the darker uterine side. Each smallest division equals 1 mm.

PLATE II

FIG. 6. Second stage larva. Note the structural outline of the digestive tract of this larva is very similar with the larva shown in figure 7.

FIG. 7. Larva released from cyst shown in figure 1.

RESEARCH NOTE

CROSS INOCULATIONS OF *TRYPANOSOMA AMBYSTOMAE*

Trypanosomes morphologically similar to *Trypanosoma ambystomae* have recently been found in *Taricha granulosa* (Lehmann, 1955, J. Parasitol. **41:** 552). Both the *Taricha granulosa* and the originally described host, *Ambystoma gracile,* were taken in Benton County, Oregon.

Cross inoculation experiments were attempted as an aid in clarifying the relationship between the two similar haemoflagellates. Two phases of the parasite were used: (1) 15-day NNN cultures prepared from infected *Ambystoma gracile* and (2) blood stream forms from the same host species. The vertebrate hosts were 4 adult *Taricha granulosa* which were captured in an apparently leech-free pond in Benton County, and whose blood smears showed no trypanosomes. Previously, 62 *Taricha granulosa* from the same pond were examined without finding haemoflagellates (Lehmann, 1954, J. Parasitol. **40:** 656–659). Citrated whole blood in the amount of 0.2 ml or 0.1 ml of culture material was injected subcutaneously into the experimental animals; 2 animals received the whole blood while the remainder were injected with culture material.

Injection of the culture forms of *Trypanosoma amystomae* produced an infection in both of the *Taricha granulosa;* in one animal a light infection was seen 12 days after inoculation, while the first haemoflagellate was noted in the other animal on the sixteenth day. The trypanosomes were few in number and small (9.6 microns in length). Aside from the diminutive size, the parasites resembled the small slender forms described by the writer (Lehmann, 1954, J. Parasitol. **40:** 656–659).

The course of infection was followed for a period of 3 weeks and the study was terminated by the accidental death of both hosts. During the 3-week period, there was neither an increase in the number of the parasites nor in the size of the individuals.

Injections of whole blood containing *Trypanosoma ambystomae* failed to produce an infection in the remaining 2 *Taricha granulosa;* the animals were examined for 29 days.—DONALD L. LEHMANN, *Department of Zoology, College of the Pacific, Stockton, Cal.*

CYRTOSOMUM PENNERI N. SP. (OXYUROIDEA; ATRACTIDAE)

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The genus *Cyrtosomum* was erected by Gedoelst (1919) to receive an atractid nematode from a North and Central American iguanid, *Sceloporus undulatus* (Daudin). The name proposed for the new species was *Cyrtosomum scelopori* Gedoelst, 1919.

Bravo-Hollis (1942) redescribed *C. scelopori* from another host, *Ctenosaura acanthura* (Shaw), a large herbivorus iguanid of Mexico. Recently, the present author obtained specimens, which have been identified by the author as *C. scelopori,* from 3 other large herbivorus iguanids. Two of these are native to the southwestern deserts of the United States, *Dipsosaurus d. dorsalis* (Baird and Girard) and *Sauromalus obesus* (Baird). The third, *Ctenosaura hemilopha* (Cope), is found in Mexico.

From several other North American iguanids, forms which differ sufficiently from *C. scelopori* to be named a new species were recovered. The name *C. penneri* n. sp. is proposed for this new species in honor of Dr. Lawrence R. Penner of the University of Connecticut.

The major portion of the material used in this study was from the personal collection of Dr. Clark P. Read. The remainder was obtained from specimens in the Herpetology Collection at the University of California at Los Angeles.

The following description of *C. penneri* n. sp. is based on alcohol-fixed specimens which were cleared in lacto-phenol. Thirty-one specimens of each sex were studied in detail. All measurements are in millimeters. For each measurement, the range is in parentheses preceded by the mode and followed by the mean.

Cyrtosomum penneri n. sp.

Diagnosis: Atractidae Travassos, 1919. Body more or less cylindrical along most of its length, tapering toward the extremities. Anterior extremity bluntly rounded; mouth terminal, surrounded by 6 inconspicuous lips. Arranged symmetrically around the mouth are 8 papillae and 2 amphids: 1 prominent knobbed papilla on each of the 2 sub-dorsal and 2 sub-ventral lips, 1 papilla and 1 amphid on each lateral lip, 1 medio-dorsal and 1 medio-ventral papilla. Esophagus divided into anterior muscular portion and posterior glandular portion, terminating in a prominent bulb with grinding apparatus. Nerve-ring at junction of esophageal parts. Excretory pore opens just anterior to esophageal bulb.

Male: 1.65 (1.21–1.76) 1.57 long, 0.108 (0.088–0.176) 0.129 wide. Esophagus 0.418 (0.341–0.462) 0.374 long: muscular portion of esophagus 0.231 (0.220–0.286) 0.259 long, glandular portion 0.154 (0.105–0.176) 0.149 long. Esophogeal bulb 0.066 (0.055–0.088) 0.071 long by 0.088 (0.066–0.099) 0.080 wide. Cloaca 0.077 (0.066–0.099) 0.078 from posterior extremity. Two spicules of unequal length: short spicule 0.099 (0.088–0.116) 0.099 long by 0.006 (0.005–0.007) 0.006 wide, long spicule 0.121 (0.110–0.138) 0.121 long by 0.007 (0.006–0.009) 0.007 wide. Six pairs of caudal papillae: 2 precloacal subventral pairs; 4 postcloacal pairs, 1 lateral, 2 ventral, 1 latero-dorsal.

Female: 1.65 (1.21–1.87) 1.57 long, 0.110 (0.088–0.187) 0.142 wide. Esophagus 0.429 (0.330–0.462) 0.401 long: muscular portion of esophagus 0.264 (0.187–0.286) 0.250 long, glandular portion 0.143 (0.110–0.187) 0.151 long. Esophageal bulb 0.077 (0.061–0.088) 0.074 long by 0.077 (0.061–0.099) 0.077 wide. Anus 0.110 (0.088–0.154) 0.119 from posterior extremity. Vulva 0.110 (0.078–0.154) 0.113 from anus and 0.264 (0.198–0.308) 0.239 from posterior extremity. Embryos present in uterus.

Type Host: *Callisaurus draconoides gabbii* Cope (In Herpetology Collection at the University of California at Los Angeles.)

Received for publication June 11, 1956.

Location: Cecum.
Type Locality: Harper's Dry Lake, San Bernardino County, California.
Type Specimens: Holotype: Male. Allotype: Female. U. S. National Museum Helmin. Coll. No. 38166.
Other Hosts: *Sceloporus occidentalis biseriatus* Hallowell; *S. graciosus vandenburgianus* Cope; *Petrosaurus* sp.

In the possession of spicules of unequal length and 2 pairs of pre-cloacal papillae instead of 3 pairs, *C. penneri* n. sp. differs from *C. scelopori* Gedoelst, 1919.

A comparison of the *en face* view of the specimens identified by the author as *C. scelopori* with that of *C. penneri* n. sp. reveals a basic similarity in the arrangement of the lips and papillae. The arrangements of papillae differ, however, in that the 2 sub-dorsal and 2 sub-ventral lips of *C. scelopori* each bear a second, less conspicuous papilla as well as a prominent knobbed papilla as is described for *C. penneri* n. sp. Also, the medio-dorsal and the medio-ventral papillae, which are described for *C. penneri* n. sp., are absent in *C. scelopori.*

It appears that neither Gedoelst (1919) nor Bravo-Hollis (1942) were aware of a pair of lateral post-cloacal papillae located between the cloaca and the cluster of papillae on the distal portion of the tail. This lateral pair of papillae, best seen in a ventral view of the tail, is described for *C. penneri* n. sp. These papillae are also present in the specimens recovered from *Dipsosaurus d. dorsalis, Sauromalus obesus* and *Ctenosaura hemilopha.* The latter forms are considered to be specifically identical to *C. scelopori* by the author. Type material of *C. scelopori* was not made available to the author, therefore, no comparative study could be made.

Considerable variation in the number and arrangement of the male caudal papillae appears in specimens of *Cyrtosomum* taken from a number of different host species. Differences in the absolute and relative lengths of the spicules, as well as in the ratio of the spicules, also occurs. Some of this variation is shown in Plate II.

The author has obtained specimens from *Sceloporus clarkii* Baird and Girard and from *S. magister rufidorsum* Yarrow, which possess three pairs of pre-cloacal papillae. These specimens cannot be otherwise morphologically differentiated from *C. penneri* n. sp. and probably represent intra-specific variants.

Specimens from *Gambelia w. wislizenii* (Baird and Girard) and *G. w. silus* (Stejneger) possess two pairs of pre-cloacal papillae and one pair of adanal papillae. These latter forms, unlike the forms from *S. clarkii* and *S. magister rufidorsum,* can be further distinguished from *C. penneri* n. sp. on the basis of spicule character. The ratio of the spicules is much greater in specimens recovered from *Gambelia* hosts than in specimens described as *C. penneri* n. sp. from other iguanid hosts.

The specimens from *Gambelia* hosts may represent a third species in the genus *Cyrtosomum* or they may be, as is suggested above for specimens from *S. clarkii* and *S. magister rufidorsum,* intra-specific variants of a single diversified species. The final answer to this problem will come probably with experiments designed to elucidate host-induced morphological variation.

In his revision of the family Atractidae, Skrjabin (1948) removed the genera *Cyrtosomum* Gedoelst, 1919 and *Monhysterides* Baylis and Daubney, 1922 from the Oxyurata and placed them in the Spirurata. Skrjabin placed the emphasis for his revision on the character of the esophageal bulb and on the host distribution

of the parasites. In justification for his removal of the genera *Cyrtosomum* and *Monhysterides* from the Oxyurata, Skrjabin noted a lack in both genera of a well defined esophageal bulb with a valvular apparatus. While this feature is lacking in certain species of the genus *Monhysterides,* a well defined esophageal bulb with valvular apparatus does occur in the genus *Cyrtosomum;* therefore, the revision of the Atractidae as suggested by Skrjabin must be rejected, at least in part.

The obvious congeneric relationship of both equal- and unequal-spiculed forms in *Cyrtosomum,* weakens the argument that spicule equality or disparity can be used as a generic criterion in the Atractidae, as suggested by Gállego-Berenguer (1947).

ACKNOWLEDGMENTS

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Explanation of Plate I

Cyrtosomum penneri n. sp.

a. Male: caudal region, ventral view.
b. Male: caudal region, lateral view.
c. Male: anterior region, lateral view.
d. Male: adult.
e. Female: *en face* view.
f. Female: adult.

Explanation of Plate II

a. *Cyrtosomum scelopori* Gedoelst, 1919 from *Sauromalus obesus* (Baird).
b. *C. scelopori* Gedoelst, 1919 from *Ctenosaura acanthura* (Shaw) after Bravo-Hollis (1942).
c. *C. scelopori* Gedoelst, 1919 from *Sceloporus undulatus* (Daudin) after Gedoelst (1919).
d. *Cyrtosomum* sp. ? from *Gambelia w. wislizenii* (Baird and Girard).
e. *Cyrtosomum* sp. ? from *Sceloporus clarkii* Baird and Girard.
f. *C. scelopori* Gedoelst, 1919 *en face* view.

PLATE I

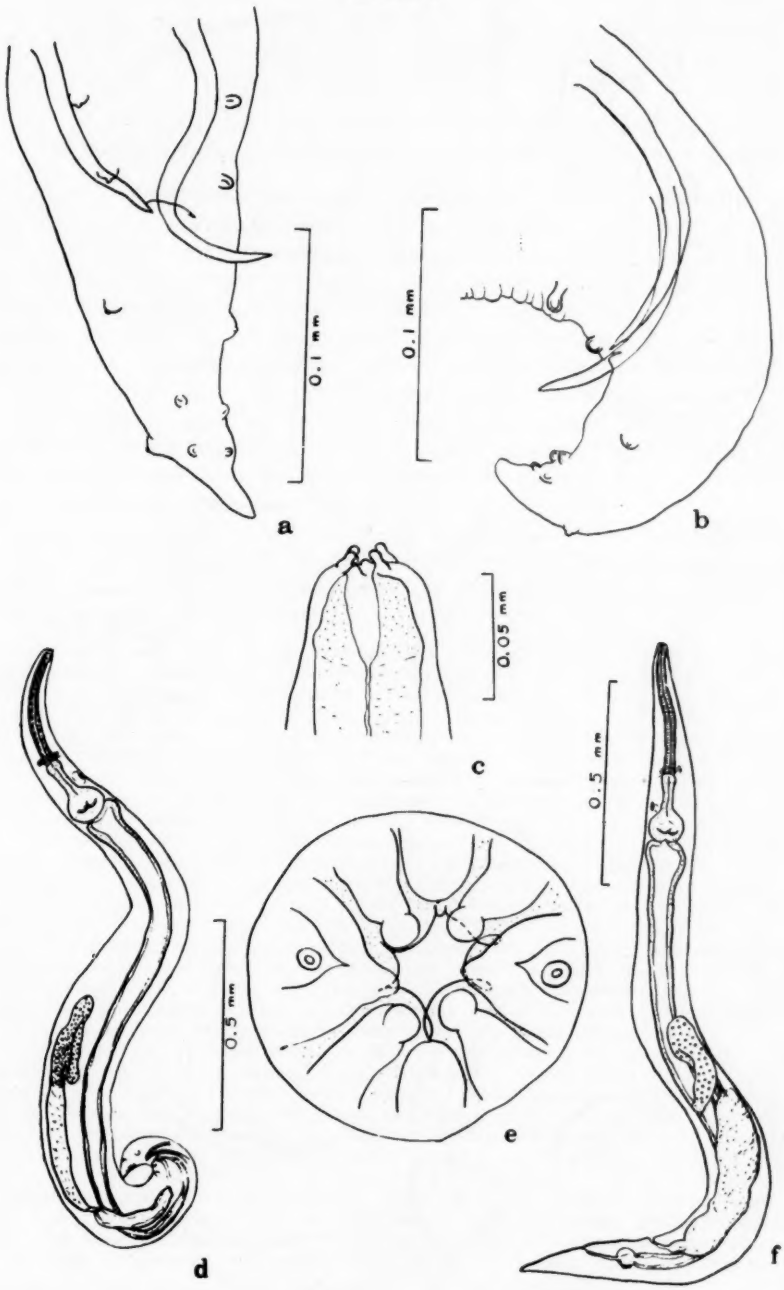
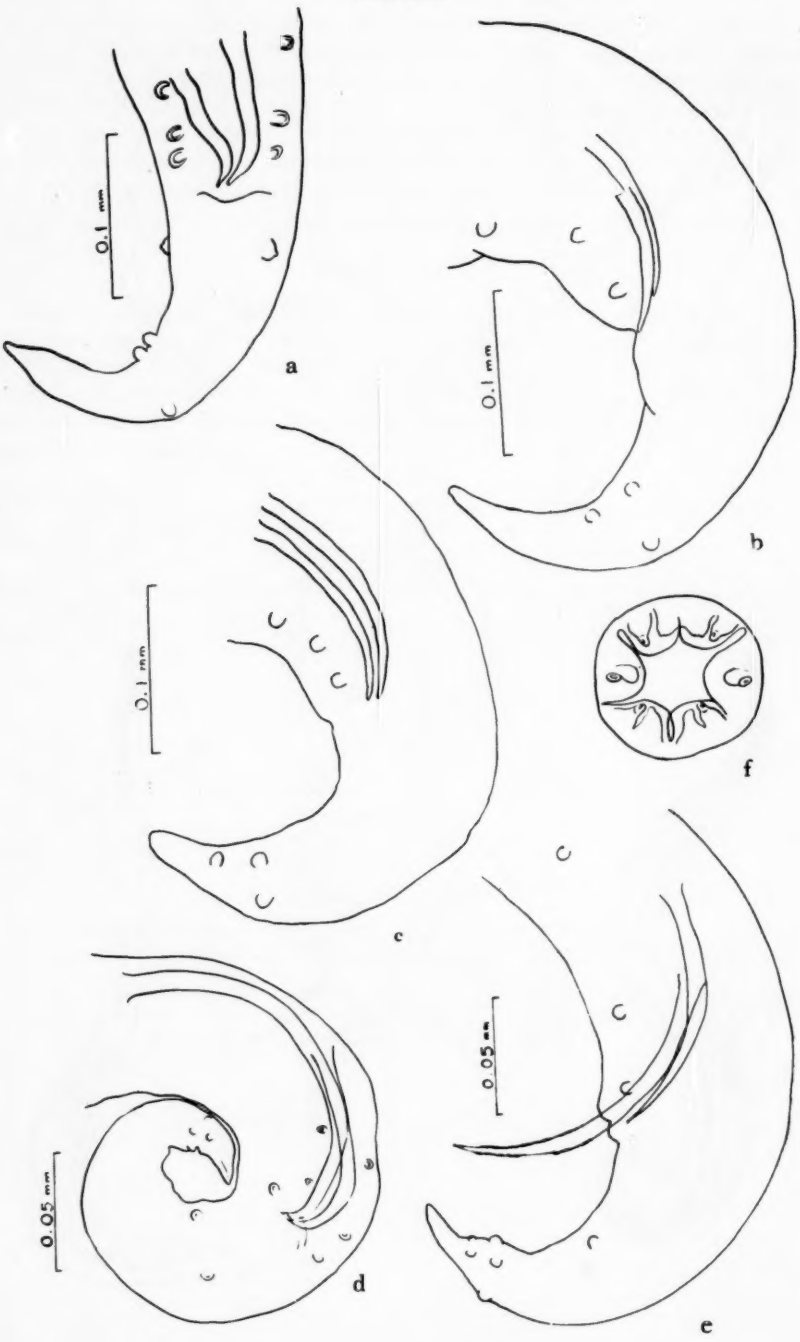


PLATE II



TWO CASES OF *RAILLIETINA* INFECTION IN INFANTS IN THAILAND, WITH A DISCUSSION OF THE TAXONOMY OF THE SPECIES OF *RAILLIETINA* (CESTODA) IN MAN, RODENTS AND MONKEYS

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Three well-preserved specimens of tapeworms of the genus *Raillietina* were obtained by the junior author from children in Bangkok. One worm was passed spontaneously from a 2-year-old child in a liquid stool, without any drug having been administered. This child was the son of a janitor at the Siriraj Hospital and lived in the employee quarters of the hospital, in an urban area. The worm was immediately preserved in 10% formalin. The other 2 worms were spontaneously passed some time previously by a 5-year-old child who was a private patient of a pediatrician in the city. All 3 worms were preserved in formalin, and were in a very good state of preservation when stained and mounted. They were also all remarkably similar both in size and in anatomical features. Since both of the children who harbored the worms lived in urban areas, the most likely reservoir host is a commensal rat of the genus *Rattus*.

Since these worms are in a much better state of preservation than those previously described from man in the Far East, and since the taxonomic status is uncertain, the following detailed description of them is given. (Measurements, unless otherwise stated, are in microns.)

Description. Length 250 to 260 mm, with maximum width, near posterior end, of 2.3 to 2.8 mm. Scolex 330–340 in diameter, not clearly demarcated from neck. Rostellum 125–130 in diameter, armed with double crown of 80–82 hooks, arranged with bases of roots at same level. Long hooks 23.5 to 24 long, short ones 20–21, shaped as shown in Figure 3*a* when separated and seen in side view, but appearing very slender in intact rostellum, with long, narrow roots about 2 in diameter, expanded at base to width of 4.5 to 5 in transverse diameter. A collar of spines 6 to 8 long behind rostellum. Suckers about 120 long, 90–100 broad, and 80 deep, apparently unarmed although refringence of muscle fibers is suggestive of spines. Neck 220 in narrowest width. First evidence of segmentation about 2 mm from anterior end.

About 30 mm from anterior end, segments about 1.3 to 1.5 mm wide, with fully developed testes. Female system mature in segments about 1.7 to 1.9 mm wide and 400 to 450 long, 40 to 50 mm from anterior end. Egg capsules first evident about 80–100 mm from anterior end, in segments measuring about 2.4 mm wide and 800 long. Towards end of strobila segments about 2 to 2.4 mm long and 2.2 to 2.8 mm wide. Detached segments become barrel-shaped, and evidently lose some egg capsules; one such measures 2.2 mm long and 1.4 mm wide. Genital pores unilateral, on right side, rarely with one on left side, situated very near anterior border of segments, at end of anterior 1/4 to 1/5 in young mature segments, but at end of first 1/10 in ripe segments. Genital atrium in mature and ripe segments about 40 deep, with the cirrus pouch and vagina opening into it independently, the latter immediately behind the former.

Cirrus pouch gourd-shaped, 160–170 by 80 in segments where first fully developed, 175–195 by 78–82 throughout rest of worms, directed antero-medially, passing lateral nerve except in old segments where pushed to more lateral position by uterine capsules. Internal vas deferens straight in distal part of pouch, with one or two loops in proximal wider part. External vas deferens very convoluted, extending to middle of segment near its anterior border. Testes confined to middle portion of segment, not reaching ventral excretory vessel on either side, though extending nearer it on aporal side. Testes number 38 to 54, 12 to 24 poral and 20 to 34 aporal, with one or two posterior to female organs. Fully mature testes about 50 to 70 in diameter.

Vagina opens into genital atrium immediately behind cirrus pouch, passes between the excretory ducts, continues in almost straight line, sometimes with one or two small kinks, to near middle of segment. Here it turns posteriad to pass dorsally between the two ovarian

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lobes, where joined by oviduct. Distal portion of vagina slightly enlarged for about 200, with diameter of about 20, then narrowing before enlarging to form oval or pyriform seminal vesicle about 200 long and 60 to 80 in diameter. In older segments this often followed by one to several additional smaller bulges of variable size filled with sperm. After vagina is joined by oviduct it forms dilation about 80 long and 40 broad, the oötype complex. Posteriorly vitelline duct proceeds in postero-ventral direction to join vitelline gland. Ovary bilobed with fairly broad isthmus, about 320–440 across, the lobes in fully ripe, mature ovary about 160–180 antero-posteriorly and 160–200 transversely, without finger-like lobes. Vitelline gland immediately behind ovary at midline of segment, of somewhat irregular shape, only slightly lobed if at all, measuring 125–130 antero-posteriorly and 140–180 transversely.

Uterus breaks up into about 300 egg capsules, at first polygonal, filling segments in single layer between lateral nerves. Polygonal capsules about 130–180 in diameter with light-staining, clear cortical layer about, 25–35 thick, and deeper-staining, more granular medulla containing eggs (Fig. 2*c*). In segments where shells of eggs first clearly seen, capsules contain from 1 to 4 eggs, majority with 2 or 3. Some capsules undergo further division, so in fully ripe segments single eggs predominate, though 2 are common, and 3 rather rare. Of adjoining nearly ripe segments some have predominance of 1- and 2- egg capsules, others a majority of 3-egg capsules. In older segments capsules tend to become free-floating and independent, with no connecting parenchyma, and lose sharp differentiation between cortex and medulla (Fig. 2*a*) and frequently become elongated (Fig. 2*b*). Capsules, even when free-floating, oriented so elongate eggs lie with long axis in dorso-ventral position or nearly so, eggs thus appearing round or oval. In capsules freed from segments eggs seen to be spindle-shaped, 100–125 long and 38–42 in diameter (Fig. 2*a*). Type specimens deposited in U. S. Nat'l Museum (Nos. 55252 and 55253).

DISCUSSION

There has been great confusion and much difference of opinion concerning the specific relationships of various specimens of Raillietina of human origin, not only to each other, but also to more or less similar forms found in rodents or monkeys in the same general regions. Joyeux and Baer (1940) placed human species of *Raillietina* in three general groups—those from the Madagascar region, those from the Oriental region, and those from tropical America, although they remarked that the two Old World groups were not easily distinguishable. As long ago as 1929, Joyeux and Baer expressed the opinion, now generally accepted, that the human cases should be regarded as accidental infections with worms normally parasitic in other mammals of the region. It is unfortunate, but to be expected, that all the worms hitherto recovered from man, after being passed with the feces, have been either incomplete or in a poor state of preservation. In this respect the worms herein described are exceptional since they are not only complete and well preserved, but have been very carefully fixed and stained.

In the Old World, specimens assigned to the genus *Raillietina* have been obtained from man in several small islands in the Madagascar region—Comoros, Mauritius, Nossi Bé (see Joyeux and Baer, 1929); southeast Africa (Clapham, 1947); Russian Turkestan (Linstow, 1901); Bangkok (Leuckart, 1891); Manila (Garrison, 1911); Formosa (Akashi, 1916; Joyeux and Baer, 1929, and others—see Miyagawa, 1950, bibliography); Batavia (Bonne and Mreyen, 1950); and Australia (Baer and Sandars, 1956). To these worms have been assigned six different names—*madagascariensis* (Davaine, 1870); *asiatica* (Linstow, 1891); *formosana* (Akashi, 1916); *garrisoni* Tubangui, 1931; *davainei* (Dollfus, 1939–40); and *celebensis* (Baer and Sandars, 1956);—although some workers consider all of these to be synonyms of *madagascariensis*. Baer (1956) identified 2 tapeworms collected from man in Mauritius in 1934 as *Inermicapsifer arvicanthidis*, a species also recorded from man and rodents in Africa and from man in Cuba, and concluded that Davaine's original *Taenia madagascariensis* was the same species, thus making *arvicanthidis* a synonym of *madagascariensis* and removing the latter from the genus Raillietina

altogether. Although possible, we do not believe there is adequate justification for this, and adhere to the view expressed by Baer and Sandars (1956) that it is impossible to establish the identity of *madagascariensis*. The name should, therefore, be dropped from future use. In the New World Dollfus (1939–40) recognized eight species from man in South America and Cuba, but most parasitologists now believe that all the South American forms from man belong to one species, *demerariensis,* and those reported from Cuba have proved to be members of the genus *Inermicapsifer,* which closely resembles *Raillietina* except in having an unarmed scolex.

The finding of worms in rats (*Rattus*) and other rodents in the Old World, and in howler monkeys and various rodents *other* than rats in the New World, has only complicated the picture. Meggitt and Subramanian (1927) recognized 8 species and 1 subspecies in rodents in southeast Asia alone; since then a number of other species [*formosana, garrisoni, sinensis* (see Hsü, 1935), *and coreensis* (see Honda, 1939)] have been described from rodents in that region, and there is also one species, *R. lateralis,* from an insectivore in Sumatra. In the Madagascar region no *Raillietina* has been reported from any of the islands (Mauritius, Comoros, Nossi Bé) where worms from man have been found, but Joyeux and Baer (1936) found 1 rat of 400 examined in Madagascar itself infected with a *Raillietina* (*R. murium*) which differs very markedly from the worms of human origin, although Baer and Sandars later (1956) synonymized it with *R. celebensis,* to which they referred all the worms from man in the Oriental Region. Another African species is *R. baeri* of species of *Rattus* in West Africa (Joyeux & Baer, 1929b).

There is a general consensus that *R. madagascariensis* of Garrison (1911) from man in the Philippines is to be considered identical with *R. garrisoni* Tubangui, 1931, of rats in those islands, and that *R. formosana* of man in Formosa (Akashi, 1916) and from rats in Formosa and Japan (Narihara, 1936; Miyazaki, 1950) are identical. Joyeux and Baer (1929a) considered *R. celebensis* of rats in Celebes, Hanoi, and Rangoon as identical with *formosana,* but these authors (1935) retained *garrisoni* as a separate species, *near* to *celebensis.* The Japanese workers, however, have not hesitated to synonymize *all* these worms with *madagascariensis* and have even included *demerariensis* of the New World. Lopez-Neyra (1954) considered as one species (*madagascariensis*) all the worms from man in the Madagascar region, the human species of Inermicapsifer from Africa and Cuba, and *Raillietina bakeri* Chandler, 1942, of North American squirrels and cotton rats. He placed the human forms from the Philippines and Japan and most of the rodent forms from southeast Asia in another species (*formosana*). In a third species, *celebensis,* which he placed in a separate genus, Meggittia, he included with Janicki's (1902) *celebensis* the specimens from man in Turkestan and Bangkok, described by Linstow (1901) and Leuckart (1891) respectively.

The situation in the New World is equally uncertain. Daniels (1892) described 2 specimens from a child in British Guiana under the name *R. demerariensis.* They lacked scolices, but differed from *madagascariensis* of the Old World in the larger number of testes, larger cirrus pouch, and more numerous eggs per uterine capsule. In 1938, Leon obtained a number of specimens from man in Ecuador, which Dollfus (1939–40) considered as 5 distinct species, but all of which Joyeux and Baer (1940) considered as *demerariensis,* a view which has been generally accepted. Subsequently Baylis (1947), Joyeux and Baer (1949), and Perkins (1950) described similar worms from howler monkeys (*Alouatta*) in the

Guianas. Joyeux and Baer considered their worms to be *demerariensis,* and thought the monkey to be the normal host, but Baylis and Perkins described their specimens under separate specific names. The chief difference from *demerariensis,* shared by both of these, is the number of testes (110–130), about twice the number reported for *demerariensis.* All the scolices described from either man or monkeys in the New World have from 150 to over 200 small rostellar hooks, usually between 14 and 20 microns in length. Baer and Sandars (1956) state that *Raillietina halli,* described from a rodent, *Capromys pilorides,* in Cuba, is identical with *demerariensis* as redescribed from monkeys by Joyeux and Baer, 1949.

Cameron and Reesal (1951) described a *Raillietina* from hystrichomorph rodents in Trinidad, which had only 28 to 32 testes, and rostellar hooks only 11 microns long. They considered that these worms, together with all those from man and monkeys in America, probably represent a single species, but to prevent confusion, pending further study, they tentatively gave their rodent parasite a new variety name (*R. demerariensis* var. *trinitatae*). Baer and Sandars (1956) raised this to a species. Stunkard (1953), however, described a *Raillietina* from another hystrichomorph rodent in Venezuela which, he said, was very similar to and probably identical with those studied by Cameron and Reesal, but which he placed in the species *demerariensis.* This worm has 26 to 46 testes and rostellar hooks 11 microns long.

If these worms from rodents are admitted to identity with *R. demerariensis* there would seem to be no sound reason for excluding *R. bakeri* Chandler, 1942, found in North American squirrels and later reported from cotton rats by Baylis, 1945, and by Harkema and Kartman, 1948. This species has 30 to 40 testes, and rostellar hooks 20 to 22 microns long. Lopez-Neyra (1954) actually *does* include it. If, however, these worms, with or without *bakeri,* are included in *R. demerariensis,* there is no longer adequate reason for separating the American forms from the Old World forms, since all the criteria used to separate them—number of testes, size and number of rostellar hooks, length of cirrus pouch, number of egg capsules, and number of eggs per capsule—break down in one or another of the various forms in the Old or New World that would have to be included. There is *less* difference between the various Old World forms from man and rodents, scattered through the Madagascar region, Africa, Asia, and the East Indies, than there is among the New World forms. If we follow the same logic as applied to the American worms we must not only consider all of the Old World forms from man and rodents as constituting a single species [as Narihara (1935) and Miyazaki (1950) have done] but we are inevitably led to the conclusion that all the rodent and primate worms in the whole world probably belong to a single species. *R. madagascariensis.*

Helminthologists have shown no such tendency to lump together species of other tapeworm genera (e.g., *Inermicapsifer, Oöchoristica, Bertiella, Hymenolepis, Taenia*). The recent demonstration of the existence of more than one species in *Echinococcus* might be a warning that too much "lumping" should be avoided. In our opinion the propriety of "lumping" is open to serious question in the case of the mammalian species of *Raillietina.* Within the described forms there are extreme differences in number of testes (28–32 to 110–130), number of rostellar hooks (60 to over 200), length of hooks (11 to 26 microns), length of cirrus pouch (80–90 × 50 to 200 to 300 × 90–100 microns), number of egg capsules (38–55 to

300–400), number of eggs per capsule (1–3 to 8–15), and size of eggs (50–64 × 19–23 to 100–115 × 40–42 microns).

There are also differences in the shape of the rostellar hooks. Although this character is considered of great importance in separating species in some genera, e.g., *Taenia* and *Hymenolepis,* it has been largely neglected in this genus, and often not described at all. It is impossible to ascertain the true shape of the hooks unless the rostellum is mashed and the hooks more or less isolated. Before such treatment, even in flattened scolices, the hooks on our worms conformed closely to the shapes illustrated by Lopez-Neyra (1954) in his Plate XX, Figures 2 and 6, for *R. formosana* and *M. celebensis* (see our Fig. 3, *c* and *d*). It seems fairly certain that the figures of the hooks of *Raillietina* from man in Ecuador by Dollfus (1939–40) (our Fig. 3*b*) do not represent the true shape of these hooks. That there is considerable variation in the shape as well as size of hooks in different forms of *Raillietina* from mammals is evident from the published figures (see our Fig. 3, *a-i*), and it is possible that more attention to this character might be of value in delimiting species. The hooks on our specimens are sufficiently different from those figured for *"R. madagascariensis"* (= *formosana*) in Japan by Miyazaki (our Fig. 3*f*) to raise a question as to their specific identity.

It seems probable that the main factor that has tended to make parasitologists go out of their way to tie together these various forms of *Raillietina* has been their sporadic occurrence as accidental parasites of man. Yet there is really no good reason for believing that the worms from man are necessarily related; if one species from a foreign host can successfully establish itself, the probability seems to me in favor of, rather than against, the view that other species, if given the opportunity, might do likewise. The human intestine, particularly in infants, has proved itself a satisfactory habitat for a remarkable variety of "foreign" tapeworms, including one or more species of *Hymenolepis, Inermicapsifer, Mesocestoides, Dipylidium, Bertiella,* and *Diphyllobothrium.*

One cogent reason for doubting the correctness of lumping together the various forms of *Raillietina* discussed above is the negative evidence of failure to find them in hosts in which they might be expected. In the Americas enormous numbers of rats of the genus *Rattus* have been examined by parasitologists, by epidemiologists working on plague, and others, and if any species of *Raillietina* occurred in them, other than as rare accidental parasites, it would inevitably have come to light. Yet there is no such record. In the United States *R. bakeri* has been found in Texas only in tree squirrels; in that area it has not been found in thousands of specimens of *Rattus* or in many hundreds of cotton rats that have been examined. In the southeastern states, however, a worm which *appears* to be specifically identical is *common* in cotton rats (31% in one area in North Carolina). Worms corresponding to the descriptions by Cameron and Reese and by Stunkard of *Raillietina* in hystrichomorph rodents have been found in no other rodents in any part of the Americas. Worms corresponding closely with those found in man and monkeys in the Guianas and in man in Ecuador have not thus far been found in any rodents in those or other areas. It would appear from this that in this genus, as in other cestode genera, there is probably a considerable degree of host specificity.

In the Old World there is less convincing evidence for a multiplicity of species, since at least in the area including southeast Asia, China, Japan, Formosa, Philippines, Celebes, and Australia, the parasites, known under a variety of names, are

all apparently common parasites of rats the genus *Rattus*. (Thus far, however, *R. coreensis* Honda, 1939, of *Apodemus agrarius* in Korea has not been found in species of *Rattus*). Since *Rattus* is readily transported in ships it might be expected that a tapeworm harbored by it would be widely distributed to islands and seaports, and these are the places where these worms have been found. The situation in the Madagascar region is less certain. Apparently no *Raillietina* have thus far been reported from rats or other rodents in the small islands (Nossi Bé, Mauritius, Comoros) where human infections have been found, and Joyeux and Baer (1936) found only 1 rat in 400 that they examined on Madagascar infected with a *Raillietina,* and this differed so markedly from the worms from man (240–250 hooks, 14 to 16 testes, and other differences) that they felt compelled to consider it a new species.

It seems to us that the lumping together of all these worms, in the present state of our knowledge, is likely to cause more confusion than simplification. Not only is it possible that different species of rodents in different geographical areas may harbor worms that are specifically distinct, but also that single genera, e.g., *Rattus,* may harbor different species of worms not only in different geographical localities, but even in the same locality. Joyeux and Baer (1940) called attention to the fact that we know nothing of the genetic constitution of tapeworms and have no sound basis for determining specificity. The problem is complicated by the existence of self- as well as cross-fertilization and development in different intermediate as well as definitive hosts, as well as by such factors as age of host, nutrition, number of worms, previous exposure to infections, concomitant infections, etc.

The worms here described are the best preserved and most complete of any thus far described from man. In the present state of confusion concerning *Raillietina* from man and rodents in the Oriental region, however, they cannot confidently be given any of the previously used names, even though it is not likely that they constitute a new species. According to the views of particular authors concerning the identity of various forms they might be called *madagascariensis, formosana,* or *celebensis.* They are probably specifically identical with the worms from which Lopez-Neyra's (1954) figures of *"R. formosana"* and *"Meggittia celebensis"* were drawn (Plates XIX, XX, XXVII and XXVIII). As far as we can judge, these worms were collected by Meggitt from rats in Rangoon. Not only the appearance of the scolex, and of the mature and ripe segments, but also the shape of the rostellar hooks as seen in front view, are strikingly similar.

On the other hand, our worms differ from the original descriptions of both *formosana* and *celebensis* in a number of respects. Unfortunately the scolex was absent on Janicki's original specimen from a Celebes rat, and the shape of the rostellar hooks of this species were not described by either Meggitt and Subramanian (1927) or Joyeaux and Baer (1929a). The rostellar hooks of our worms differ in shape from those figured by Miyazaki (1950) from rats in Japan (cf. Figs. 3*a* and 3*f*) which he considered identical with those found in man and rats in Formosa; these are the worms that were originally named *formosana* by Akashi (1916), but have been called *madagascariensis* by later Japanese writers.

Our worms have larger eggs (100–115 × 38–42 microns) than any form described from man or rodents except *formosana,* in which they are described as 99–110 × 46–73 microns. In all other *Raillietina* from man and rodents the size range is reported as from 34 to 60 × 20 to 45 microns. Our worms have a larger

cirrus pouch than do any of the Old World forms except the extremes reported for *R. garrisoni,* and they have a larger number of egg capsules than any except the extremes reported for *R. garrisoni* (Tubangui, 1931) and *R. formosana* (Miyazaki, 1950).

In view of these considerations it seems to us that the best course to follow is tentatively to give our worms from man in Bangkok a distinctive name until such time as the taxonomic tangle involved has been straightened out, even though it is possible, perhaps even probable, that this name will eventually become a synonym of one of those mentioned above. For the time being, therefore, we suggest the name *Raillietina* (*R.*) *siriraji,* in honor of the hospital where these worms were obtained. It is quite possible that the worm found by Leuckart (1891) in an infant in Bangkok belongs to this same species, but not sufficient details are available in the description to make this more than a surmise.

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RESEARCH NOTE

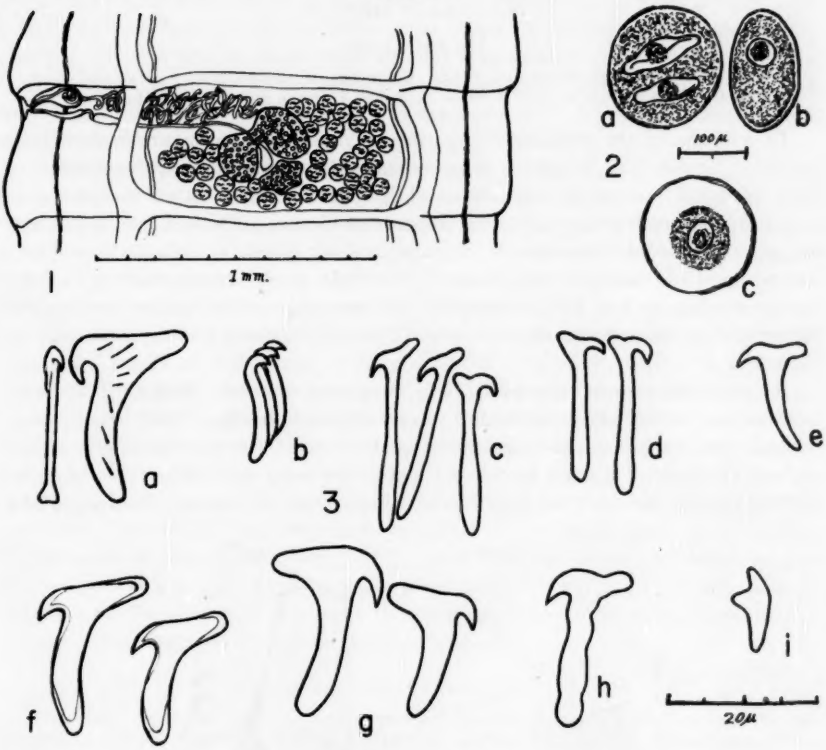
ABSENCE OF COLLAGENASE ACTIVITY IN *ENDAMOEBA HISTOLYTICA*

There is little information available on the proteolytic enzymes of *Endamoeba histolytica.* This study was undertaken to determine whether or not this protozoan produces collagenase *in vitro.*

The method of assay for collagenase activity consisted of placing small pieces of whole rat tail tendon and collagen prepared from rat tail tendon into test tubes containing amebas. Dissolution of the opaque collagen occurs in the event that collagenase is present or is produced. The assay was conducted against 3 strains of *E. histolytica* (NRS, UC, and HUS-100) under bacteriostatic conditions using a technic described earlier (Nakamura, Proc. Soc. Exper. Biol. Med., **89:** 680–682, 1955) and also under stock culture conditions containing actively growing associated bacteria. In performing the test for collagenase, the collagen was introduced into freshly transferred ameba cultures and also into tubes containing pooled amebas from twenty 48-hour cultures. All tubes were incubated at 37° C and observed for 5–7 days before discarding. The initial *p*H of the media was 7.2 and the final *p*H was 6.5–6.8. Control tests consisted of culture media containing small amounts of collagenase (prepared from *Cl. histolyticum*).

The collagen was dissolved in 12–24 hours in control tubes which contained collagenase, indicating that the medium is not inhibitory to the enzyme. None of the tubes containing amebas exhibited collagenase activity. This indicates the absence of collagenase in *E. histolytica* cultivated *in vitro* by our particular techniques.—MITSURU NAKAMURA* AND LEROY KLEIN, *Departments of Microbiology and Biochemistry, Boston University School of Medicine, Boston, Mass.* (* *Present address, Department of Bacteriology, Montana State University, Missoula.*) This work was supported (in part) by the Office of the Surgeon General, Department of the Army, Contract Nos. DA-49-007-MD-711 and DA-49-007-MD-287.

PLATE I



EXPLANATION OF PLATE

FIGURE 1. Mature segment of *Raillietina siriraji.*

FIGURE 2. Egg capsules of *R. siriraji; a* and *b* fully ripe capsules, floating free in detached segment; *c,* immature capsule from segment in which capsules have thick hyaline outer wall, not clearly differentiated in fully ripe capsules.

FIGURE 3. Rostellar hooks from various species of Raillietina from man, monkeys and rodents, drawn to scale, after various authors.

a. R. siriraji. The dark lines represent attachment of muscle fibers.
b. R. quitensis (=*demerariensis*), after Dollfus (1939–40).
a. R. formosana, after Lopez Neyra (1954), Pl. XX.
d. Meggittia celebensis (=*R. celebensis*), after Lopez-Neyra (1954), Pl. XX.
c. R. multitesticulata, after Perkins (1950).
f. R. madagascariensis (=*formosana*), after Miyazaki (1950).
g. R. murium, after Joyeux and Baer (1936).
h. R. bakeri, after Chandler (1942).
i. R. demerariensis var. *trinitatae,* after Cameron and Reesal (1951). (This does not conform to the type of hook found in Raillietina; it looks like the head of a Raillietina hook with the stem broken off).

GREGARINE PROTOZOA OF SILVERFISH (INSECTA: THYSANURA) FROM CEYLON AND ENGLAND, WITH THE RECOGNITION OF A NEW GENUS

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Department of Parasitology, London School of Hygiene and Tropical Medicine

In a study of the cephaline gregarines of domestic Thysanura collected by me from Ceylon and England, I have found the following host-distribution of these parasites: *Gregarina lagenoides* (Léger, 1892) in *Lepisma saccharina* in England; *Lepismatophila thermobiae* Adams and Travis, 1935, in *Lepisma saccharina* and *Thermobia domestica* in England and in *Acrotelsa collaris* in Ceylon; *Colepismatophila watsonae* Adams and Travis, 1935, in *Acrotelsa collaris* in Ceylon; *Lepismatophila* sp. and *Colepismatophila* sp. (probably a new species with spore-filaments distinctly shorter than those of *C. watsonae*) from *Peliolepisma calva* in Ceylon.

A gregarine closely resembling both *Gregarina aciculata* Bhatia, 1938, from *Lepisma saccharina* (?) in India, and *G. ctenolepismae* Lindsay, 1939, from *Ctenolepisma longicaudata* in Australia, was found by me in the anterior region of the mid-gut and in the 2 mid-gut sacculi of *Peliolepisma calva* in Ceylon. The epimerite of this gregarine is seen to be rather labile (Figs. 1 and 2), varying from a peg-like

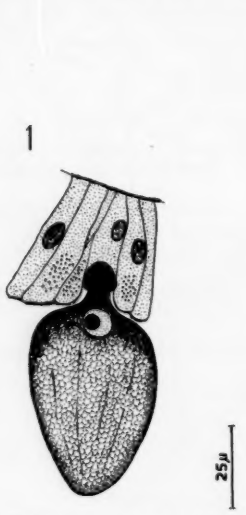

FIGURE 1. *Garnhamia aciculata.* Trophozoite attached to mid-gut epithelium of *Peliolepisma calva.*

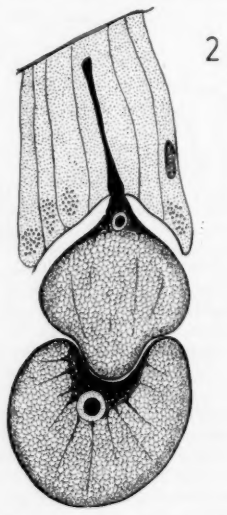

FIGURE 2. *Garnhamia aciculata.* Trophozoites in syzygy.

or papillate structure as in *G. ctenolepismae,* especially in the younger forms, to an acicular structure as in *G. aciculata.* In other respects this form is indistinguishable

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from both the Indian and the Australian species. It is also almost certain that the Indian form, *G. aciculata,* did not come from *Lepisma saccharina* which has no mid-gut sacculi. Cornwall (1915) originally described this gregarine from a lepismatid with "8 to 10 large sacculi," which insect he doubtfully regarded as *Lepisma saccharina.* He was dealing most probably with a species of *Ctenolepisma,* which is fairly common in India and has well-developed sacculi. These considerations lead me to regard *Gregarina ctenolepismae* as a synonym of *G. aciculata,* as there are no satisfactory characters on which the two forms could be validly separated.

A further important consideration arises from the fact that *G. aciculata* (syn: *G. ctenolepismae*) is a dicystid type of gregarine, having only an epimerite and deutomerite and lacking a septum between deutomerite and protomerite. Indeed a protomerite is absent, unless the somewhat hyaline anterior end of the deutomerite be regarded as one. This species is therefore a non-septate cephaline gregarine and cannot rightly belong to the genus *Gregarina.* It is also not possible to include it among members of the dicystid family Lecudinidae, because an important diagnostic feature of that family is the absence of syzygy, whereas in this species there is a distinct and even precocious syzygy. Nor can it be referred to the genus *Gamocystis* Schneider (Gregarinidae) which, though non-septate, has a protomerite in the early stages and shows only partial sporulation, the cysts being without the typical elongate spore-ducts. Another interesting feature, as pointed out by Lindsay (1939), is that the nucleus in this species is at the anterior end of the deutomerite, apparently tethered there by means of myoneme fibres as in *Hyalosporina cambolopsisae* Chakravarty, 1935. It is therefore proposed to erect a new genus *Garnhamia* to contain this species from India, Ceylon and Australia, and to include it for the present in the family Gregarinidae, close to the genus *Gamocystis.* The following diagnosis of the new genus is proposed:

GARNHAMIA gen. nov.

Diagnosis: Protozoa: Sporozoa: Gregarinidae: Syzygy takes place while primite is still attached to mid-gut epithelium of host. Epimerite papillate to acicular. Non-septate. Protomerite absent. Nucleus tethered at anterior end of deutomerite. Cysts with spore-ducts. Spores ovoidal, extruded in chains. Parasites of mid-gut of Lepismatidae (Insecta: Thysanura). *Type: Garnhamia aciculata* (Bhatia, 1938) (Synonyms: *Gregarina aciculata* Bhatia, 1938; *Gregarina ctenolepismae* Lindsay, 1939).

All the above gregarines were examined and drawn in the living state in saline, and in serial sections, 5–8 microns thick, of host-guts fixed in Carnoy's or Bouin's fluid, embedded in paraffin or celloidin-paraffin (Peterfi's method) and stained in Delafield's or Ehrlich's haematoxylin and eosin or in Chlorazol Black E. Cysts and spores were recovered from host feces. The silverfish were reared in the laboratory in separate culture-jars under optimum conditions of temperature and relative humidity for each species. Further work, involving life-history studies and transmission experiments, is in progress.

I have to thank Professor P. C. C. Garnham, Department of Parasitology, London School of Hygiene and Tropical Medicine, for facilities and encouragement given. Dr. Theresa Clay of the British Museum (Nat. Hist.) and Dr. Petre Wygodzinsky of the Argentine very kindly helped in the identification of the hosts. Professor R. T. Leiper, Dr. Theresa Clay, Dr. Albert Samaraweera, Messrs. G. A. Brett and A. G. Histed of the Infestation Control Division of the Ministry of Agriculture, Fisheries and Food, Tolworth, Surrey, and the Director, Jealott's Hill Re-

search Station (I.C.I.) Bracknell, Berks., made available to me the 2 species of English silverfish used in this study.

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RESEARCH NOTE

OCCURRENCE OF *CRUZIA AMERICANA* IN TEXAS NINE-BANDED ARMADILLOS

From time to time, armadillos employed by the writer in physiological studies have been examined for intestinal parasites. On 2 occasions single specimens of nematodes of the genus *Cruzia* were recovered from the colons of adult female armadillos. One of the worms was a male and the other an immature female. Upon examination these worms appear to be *Cruzia americana.*

Cruzia americana was described by Maplestone (Rec. Ind. Mus., Calcutta **32:** 77–105, 1930) from specimens taken from the North American opossum that Canavan (Parasitology **29:** 63–102, 1929) had identified as *C. tentaculata.* Ruiz (Thesis, Sao Paulo, 105 pp, 1947) in his revision of the genus decided that *C. americana* was synonymous with *C. tentaculata.* Crites (1956, J. Parasitol. **42:** 68–72) has recently redescribed *C. americana* and pointed out numerous morphological differences between it and *C. tentaculata,* the most significant of which is the presence of 11 pairs of caudal papillae in the male of the former species. Crites feels that *C. americana* is the only species of *Cruzia* present in North American opossums.

The male worm examined by the writer has 11 pairs of caudal papillae and although it is shorter than the worms described by Crites, the internal measurements correspond nicely to those for *C. americana.* The pattern of the post-cloacal papillae varies slightly from that figured by Crites, but Wolfgang (1951, Canad. J. Zool. **29:** 352–372) has pointed out that the arrangement of the papillae is subject to some variation.

Below are the measurements obtained by the writer from the worms taken from the armadillos. Measurements are in millimeters.

Male: length, 8.3; width, 0.55; entire esophagus, 2.2; pharynx, 0.19; tubular part, 1.61; anterior bulb, 0.11; posterior bulb, 0.29. Intestinal diverticulum 0.79. Nerve ring 0.48 from anterior end. Distance anus to tip of tail 0.16. Spicules 0.81, gubernaculum 0.16 in length. 3 pairs of pre-cloacal, 3 pairs of ad-cloacal, and 5 pairs of post-cloacal papillae.

Female: length 9.5; width, 0.53; length of tail, 0.71. Ratio of tail to body length 13.4. Other measurements not recorded as worm is obviously immature.—G. D. BUCHANAN, *Department of Biology, Sam Houston State Teachers College, Huntsville, Texas.*

A NEW SPECIES OF ORIBATOID MITE FROM GUAM WITH A KEY TO THE SPECIES OF THE GENUS *NASOZETES* SELLNICK 1930 (ACARINA, ORIBATEI)

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Among mites collected on Guam in July 1946 was a species of *Nasozetes* found on the breadfruit (*Artocarpus altilis*). This mite herein described as new is closely related to the typical species, *Nasozetes sumatrensis* Sellnick (1930), and to *Nasozetes choerognathus* Willmann (1931b) the only 2 previously described species of this genus which belongs in the family Eremaeidae Willmann (1931a). The main generic character is an unusual sexual dimorphism in which the males possess a prominent anterior extension of the rostrum which is expanded into a circular disc or nose (Figs. A, E & F). This rostral expansion is much reduced in the females of *N. sumatrensis* and is entirely absent from the females of the new species (Fig. C). There are no descriptions of the females of *N. choerognathus;* therefore it is impossible to determine if this species exhibits the same type of sexual dimorphism. This condition is not known to be present in any other genus of the oribatoid mites.

The author has been able to study this mite through the courtesy of Dr. E. W. Baker, Acarologist, Entomology Research Branch, U. S. Dept. of Agriculture. The material was collected by Dr. H. K. Townes during an entomological survey of Micronesia under the auspices of the U. S. Commercial Co., Honolulu, T. H.

Nasozetes stunkardi n. sp.[1]

Description: Body thinly sclerotized, light brown or amber in color. Body size derived from measurements in microns. Length of male, based on 5 specimens, 434–454 and greatest width 293–303; length of female, based on 4 specimens, 404–414 and greatest width 278–313. Hysterosoma broadly oval and smooth. Propodosoma broader at base than long, tapering gradually to neck of nose in male and to rounded tip of rostrum in female. Line of attachment of nose of male to propodosoma indiscernible; nose short, broad with rounded corners; length of nose 49, narrowest part of nose 40. Propodosoma with well-developed, ledge-like lamellae. Interlamellar, lamellar and rostral bristles barbed. Lamellar bristles appear heavier and more distinctly barbed than others, and project about one sixth their length beyond nose. Insertions of interlamellar bristles nearer lamellae than to anterior rim of hysterosoma. Rostral bristles curve inward beyond distal end of rostrum and are inserted at anterior tips of slight rostral ridges which extend posteriorly to lamellae. Translamellae absent. Pseudostigmata posterior and ventral to anterior rim of hysterosoma. Pseudostigmatic organs with slender, straight pedicels bearing almost spherical heads which are smooth externally but appear full of granules. Pseudostigmatic organs protrude short distance from under anterior rim of hysterosoma. Proterosoma-hysterosoma suture distinct. Dorsal hysterosoma setal and porose area patterns as illustrated (Figs. A & C). Hysterosoma bears 7 pairs of short, curved dorsal setae and 1 pair caudal setae. Four pairs porose areas small and situated on each side hysterosoma. Adalar porose areas largest and reniform. Other 3 pairs circular with exception of mesonotal which are double. Ventral plate well-developed and similar to other 2 species in genus (Fig. B). Gnathosoma almost as wide as long (95 × 78). Tectopedia I and II well developed and visible from dorsal and ventral views. Apodemata I, II, III distinct; mesal ends of apodemata II & III extend to marginal rim of genital aperture. Genital plates have 4 pairs of setae; anal plates have 2 pairs. Anal plates nearly twice as

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[1] Named in honor of Dr. Horace W. Stunkard, New York University, in appreciation of his outstanding contribution in discovering the role of the oribatoid mites in the life cycle of the anoplocephaline cestodes.

long as genital plates and widely separated from them. Legs shorter than body and of ordinary structure (Fig. D). Tarsi tri-dactylous; median hook slightly larger than laterals. *Type data:* Holotype, male, on *Artocarpus altilis,* Guam, July 7, 1946; deposited in U. S. National Museum, No. 2229. Paratypes, 6 males and 6 females, same data as holotype, deposited in U. S. National Museum. Two paratypes, 1 male and 1 female, retained by the author.

DISCUSSION

The genus *Nasozetes* Sellnick may be readily distinguished from other genera of the family Eremaeidae Willmann (1931a) by the presence in the males of a bulbous anterior projection of the rostrum. The females either have this structure reduced or absent. Such an unusual and striking sexual dimorphism is not evident in any other group of the oribatoid mites. *N. stunkardi* n. sp. differs from the other 2 previously described species in this genus in a number of important characters. The most conspicuous structure, the nose of the male, is quite different in each species. In *N. stunkardi* the line denoting the attachment of the nose to the propodosoma is invisible, whereas it is distinct in both *N. sumatrensis* and *N. choerognathus* (Figs. E & F). In the Sellnick species the nose is longer (75), narrower at the base (28), and is rounded forward. In the Willmann species it is shorter (45), more broadly attached at the base (35), and has sharp corners anteriorly. A comparison of figures will best illustrate the differences. In *N. choerognathus* the lamellar bristles extend almost half their length beyond the anterior tip of the nose, while in the other 2 species these bristles project only about one sixth to one fifth their length beyond the nose. *N. choerognathus* also differs in having the whole head and part of the pedicel project out from under the anterior rim of the hysterosoma, whereas in the new species and in *N. sumatrensis* only part of the head protrudes. A comparison of the dorsal hysterosoma setae indicates specific variations. On *N. sumatrensis* these setae are so minute that they are difficult to recognize and were not figured on the original drawings of Sellnick. Six pairs of dorsal setae can be distinguished on *N. choerognathus* together with 3 additional pairs on the caudal end of the hysterosoma. *N. stunkardi* is somewhat intermediate in having 7 pairs of short, curved dorsal setae and only 1 pair of caudal setae. The shape of the gnathosoma of *N. sumatrensis* is similar to that of *N. stunkardi* but both differ from that of *N. choerognathus* which is longer (103) and narrower (69). The genital and anal plates are probably similar on all 3 species; however, Sellnick (1930) has figured only 3 pairs of genital setae on *N. sumatrensis* but it is presumed that this is an oversight since the pattern for this group of mites is obviously 4. Willmann (1931b) omitted them from his figures and description and it is assumed that the above formulae are also valid for *N. choerognathus.* An examination of figures of the 3 species indicates that the setal pattern and structure of the legs of members of this genus are so similar as to be of negligible value in the determination of species. The new species is slightly smaller than either *N. sumatrensis* (480) or *N. choerognathus* (495) but these differences may be due to individual variations which may disappear when more specimens are available for study.

The ecological niches of the 3 species are dissimilar. *N. sumatrensis* was originally collected in the region of Fort de Kock on the west coast of Sumatra. The specimens were found on galls of *Gardenia* growing at an elevation of 920 m. *N. choerognathus* was also collected on the west coast of Sumatra in a region near

Padang, which is not far from Fort de Kock. The habitat in this case was *Sphagnum* which was growing in a swampy area at an elevation of approximately 1520 m. *N. stunkardi,* on the other hand, was found on Guam on the breadfruit (*Artocarpus altilis*). The collection of species of *Nasozetes* from 2 such widely separated areas as Sumatra and Guam indicates that this very distinctive genus of oribatoid mites is probably widely scattered throughout the islands of the Malay Archipelago and Micronesia and presumably contains a number of undescribed species.

A Key to the Species of the Genus Nasozetes Sellnick 1930

1. Nose of males with rounded corners, gnathosoma almost as wide as long, lamellar hairs extending about 1/5 their length beyond nose 2
   Nose of males with sharp corners, gnathosoma ½ again as long as wide, lamellar hairs extending about ½ their length beyond the nose ..... *choerognathus* Willmann
2. Nose of males long, more than twice the width of the narrowest part, females with long narrow nose, dorsal setae very difficult to distinguish ..... *sumatrensis* Sellnick
   Nose of males short and broad, females without nose, 7 pairs of dorsal setae can be distinguished ..... *stunkardi* n. sp.

SUMMARY

Described as new is *Nasozetes stunkardi,* an oribatoid mite found on the breadfruit (*Artocarpus altilis*) on Guam, and a key to the three species in the genus is provided.

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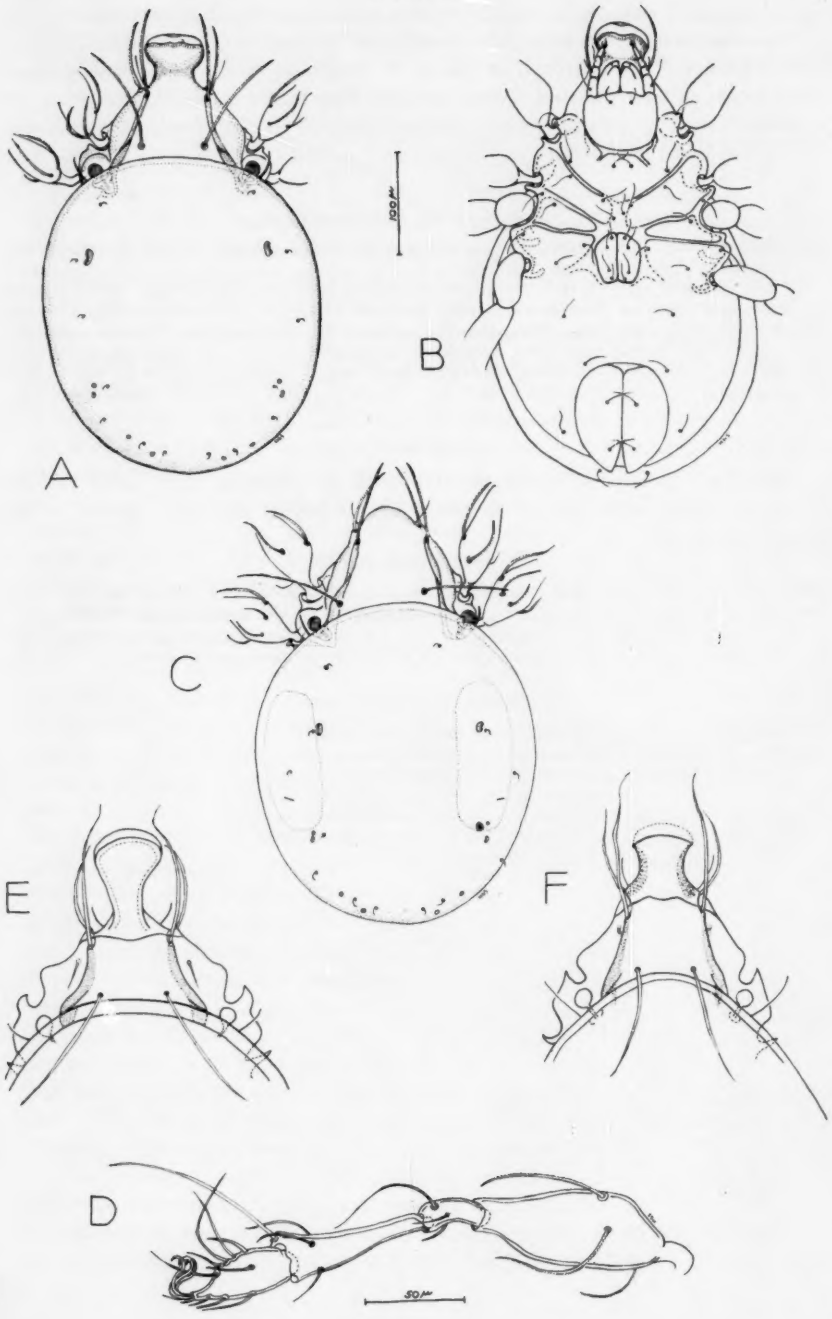
EXPLANATION OF PLATE

FIG. A. *Nasozetes stunkardi* n. sp. dorsal view, male.
FIG. B. *Nasozetes stunkardi* n. sp. ventral view, male.
FIG. C. *Nasozetes stunkardi* n. sp. dorsal view, female.
FIG. D. *Nasozetes stunkardi* n. sp. median aspect, leg I, male.
FIG. E. *Nasozetes sumatrensis* Sellnick. propodosoma, male.
FIG. F. *Nasozetes choerognathus* Willmann. propodosoma, male.
(Figs. E & F after Willmann 1931)

PLATE I



OCTOSPINIFEROIDES CHANDLERI N. GEN., N. SP., A NEOECHINORHYNCHID ACANTHOCEPHALAN FROM *FUNDULUS GRANDIS* BAIRD AND GIRARD ON THE TEXAS COAST

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Two immature female acanthocephalans were recovered from 1 of 3 Gulf killifish (*Fundulus grandis*) seined in East Bay near Gilchrist, Texas on April 9, 1956. Subsequent examination of 26 *F. grandis* (16 of which were from the same locality) from several points on the Texas coast failed to produce any further specimens. Previous examinations of 74 *F. grandis* were also negative for this parasite as were over 600 other cyprinodontid fishes from areas in which *F. grandis* occurred. Because of the distinctive nature of these worms it was concluded that a new genus and species are involved. It is hoped that other workers in the area may be able to give a more complete description after additional material has been found. A tentative diagnosis of the genus and species is given below.

The worms were relaxed in distilled water until turgid, fixed in Bouin's fixative, and stained in picrocarmine. Because of a superficial resemblance to the genus *Octospinifer* these parasites are referred to the new genus *Octospiniferoides*. In appreciation for the use of the laboratories at the Rice Institute for the school year and for the kind and stimulating advice of Dr. Asa C. Chandler, the present species is described as *Octospiniferoides chandleri*.

OCTOSPINIFEROIDES new genus

Neoechinorhynchidae with globular proboscis on which the hooks are arranged in 3 circles of 8 to 10 hooks each. Hooks small and slender with prominent roots; roots longer than thorn in all 3 circles. Proboscis receptacle thin-walled. Female genital opening nearly terminal. Male unknown. Parasitic in the intestine of brackish water fish.

Type: Octospiniferoides chandleri.

Octospiniferoides chandleri, n. sp.

Diagnosis: With the characters of the genus. Body small, not very elongate: that of immature females 1.6 and 1.8 mm long by 0.32 and 0.34 mm wide respectively. Proboscis about 0.07 mm long by 0.12 mm wide; apical organ prominent and relatively long, 0.88 mm. Hooks of smaller specimen (paratype) in 3 rows of 8 hooks each; in larger specimen (holotype) there are 10 hooks in the first and third rows and 8 hooks in the middle row. The rows of hooks were slightly irregular in their arrangement and did not appear to be evenly spaced (particularly in holotype specimen). Hooks small and fine with prominent roots in all 3 rows; length of thorn 0.024 mm in apical circle; 0.018 mm in middle circle, and 0.014 mm in basal circle; width of thorn in region of penetration of subcuticula 0.003 to 0.004 mm in all 3 circles; length of root plate 0.030 mm in apical circle, 0.024 mm in middle circle, and 0.016 mm in basal circle. Proboscis receptacle 0.15 and 0.20 mm long; wall thin, especially on ventral side of receptacle. Proboscis ganglion in postero-ventral region of receptacle; 2 prominent ganglion cells about halfway between the posterior end of the receptacle and the junction of the receptacle and proboscis.

Lemnisci equal, about twice the length of the receptacle. Giant nuclei of lemnisci in typical neoechinorhynchid pattern of 1 in one lemniscus and 2 in the other.

Female reproductive tract opening near the posterior extremity; Vagina longer than uterus. Uterine bell weakly muscular. Few nearly mature eggs in the body cavity of paratype specimen measured 0.038 by 0.014 mm. Males not observed.

Type host: Gulf killifish (*Fundulus grandis*).

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Type locality: East Bay; at Gilchrist, Texas.
Specimens: Holotype female and paratype female (No. 38197) deposited in the Helminthological Collection of the U. S. National Museum.

Octospiniferoides chandleri differs from all other members of the family Neoechinorhynchidae on the basis of the arrangement and shape of the proboscis hooks. It appears to be unique in that the basal row of hooks, as well as the more anterior rows, are provided with prominent root plates. *Octospiniferoides* is most similar to the genus *Octospinifer*. It differs from the members of this genus, however, in body shape, the nature of the receptacle wall, the position of the female genital opening, and in the shape and size of the proboscis hooks. On the basis of only 2 specimens it is impossible to assign any limits to the number of proboscis hooks, but it appears that, in contrast to other members of the Neoechinorhynchidae such as *Neoechinorhynchus, Gracilisentis,* and *Octospinifer,* the hook number of this species may not be constant.

O. chandleri brings to 3 the number of neoechinorhynchid acanthocephalans that appear to be distinctive of the Gulf of Mexico and nearby waters. *Atactorhynchus verecundus* Chandler, 1935 has been recorded from the variegated minnow (*Cyprinodon variegatus*) in Galveston Bay. Collections by the author along the Texas coast during the 1955–56 school year indicate that *A. verecundus* is a common parasite of the variegated minnow along the greater part of the Texas coast. Examination of over 700 cyprinodontid fishes further indicate a rigid host specificity since no specimens of this species were found in *F. grandis, F. similis, F. pulvereus, Lucania parva,* or *Adinia multifasciata.* The related *Mollienisia latipinna* was likewise negative.

Ward (1953) described *Floridosentis elongatus* as a distinctive neoechinorhynchid from the white mullet (*Mugil cephalus*) in the region of Miami, Florida. Cable and Quick (1954) also reported this parasite from mullet in Puerto Rico. One mature male specimen of this species was obtained from 1 of 13 mullet seined in the Laguna Madre near Port Isabel, Texas in April, 1956. No specimens of this parasite, however, were found in 55 mullet examined from more northerly points on the Texas coast. This species, then, appears to be more southerly in its distribution than either *A. verecundus* or *O. chandleri.*

Two other acanthocephalans were found in cyprinodontid fishes from the Texas coast. One immature female *Telosentis tenuicornis* was obtained from *F. grandis* at Riviera Beach. Two larval *Arhythmorhynchus duocinctus* were found in the mesenteries of one *F. grandis* in East Bay at Gilchrist, Texas. Both of these collections constitute new host records for these parasites.

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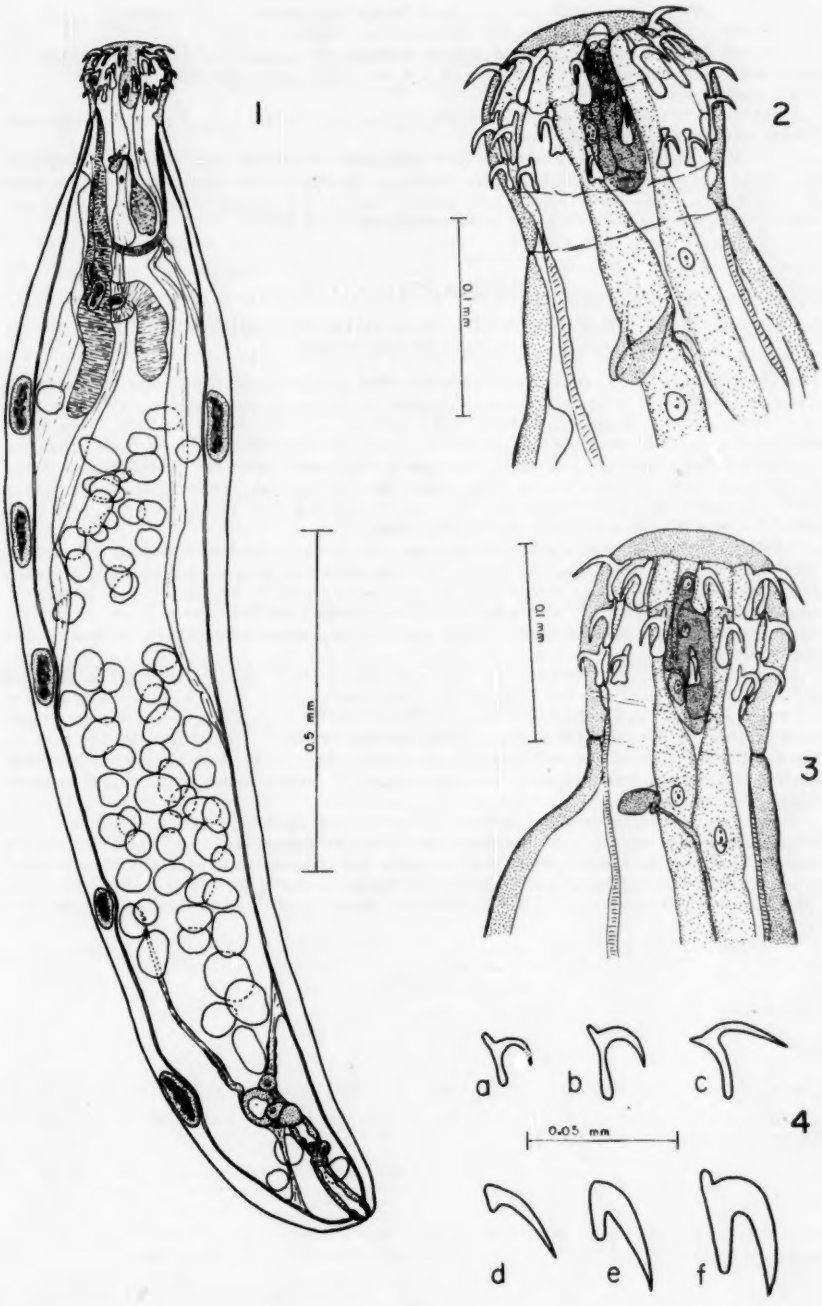
PLATE I



EXPLANATION OF PLATE

(All drawings made with a camera lucida from stained whole mounts.)

FIGURE 1. Holotype female of *Octospiniferoides chandleri*, n. sp.

FIGURE 2. Proboscis of holotype female showing arrangement of hooks and details of proboscis. Note evidence for the presence of 10 hooks in the apical and basal circle and 8 hooks in the middle circle.

FIGURE 3. Proboscis of paratype female showing same details as in Figure 2. Note hook pattern of 3 circles of 8 hooks each.

FIGURE 4. Comparison of hooks of *Octospiniferoides chandleri* with those of *Octospinifer macilentis*. a–c) basal, middle, and apical hooks of *Octospiniferoides* showing prominent roots of all 3 circles. d–f) basal, middle, and apical hooks of *Octospinifer* showing the larger and thicker thorn and the reduced roots of the middle and basal hooks.

RESEARCH NOTE

A NOTE ON THE EFFECT OF PARASITE BURDEN ON THE ACTIVITY OF FISH

There is little published information on the effect of parasite burden on the vigor and activity of fish. Thus it seems appropriate to record the following observations. Two specimens of *Lepomis gibbosus* (common sunfish), and 1 specimen of *Ambloplites rupestris* (rock bass) were used as hosts in work with *Azygia longa*. They had been obtained in the vicinity of Ann Arbor, Michigan from an area in which azygiid trematodes were not encountered and had been housed in the laboratory in a large cement tank for several months prior to use. They were maintained on a parasite-free diet of a commercial dog food preparation and were often unfed for periods of 1 to 2 weeks during the winter.

When transferred to separate glass aquaria prior to the beginning of feeding experiments, they appeared to be quite vigorous and active in avoiding reactions to netting, and in feeding activities. From the start of the experiments they were regularly fed parasite-free laboratory-raised tadpoles and guppies. Cercariae of *A. longa* from experimentally infected snails were pipetted directly into their stomachs. Until autopsy, they appeared always to be healthy and vigorously active.

When sacrificed, the livers of all 3 fish were seen to be completely occupied by encysted strigeid metacercariae. At least 500 to 600 cysts were present in each of the livers. The strigeid is identified as *Posthodiplostomum minimum centrarchi* according to the key to strigeid metacercariae of Hoffman (1955, Amer. Midland Nat. **53**: 198). In addition to this parasite, the rock bass harboured the azygiids, several *Cryptogonimus chyli*, and 3 tapeworm plerocercoids. The sunfish each harboured a single azygiid, 3 plerocercoids, and encysted metacercariae of *Clinostomum*.

So many metacercariae were packed together in each of the livers one would have been led to believe that scarcely any functional liver tissue remained. However, from their actions prior to autopsy, the fish did not appear to suffer any inconvenience from the presence over a period of several months of masses of strigeid metacercariae in their livers.—E. I. SILLMAN, *Department of Entomology & Zoology, Ontario Agricultural College, Guelph, Canada.*

ON THE MITES PARASITIZING *ANODONTA* (UNIONIDAE; MOLLUSCA)

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Department of Zoology, University of Vermont

The status of the two species of *Unionicola* that commonly parasitize mussels of the genus *Anodonta* has been uncertain ever since Wolcott's monograph of the new world *Unionicola* (1899). Wolcott stated that the two *Anodonta* parasites found in North America were the same as the two old world species. Piersig (1900) did not agree and applied new varietal names to the specimens described by Wolcott. Subsequently both Marshall (1933) and Viets (1954) regarded *U. ypsilophora* to be a Holarctic species, thus synonomizing Piersig's variety *haldemani*. Viets (1954) considered *U. intermedia* to be holarctic and synonomised the variety *wolcotti*. Marshall (1933) retained the varietal name *wolcotti* to designate the nearctic population of *U. intermedia*. Neither Viets nor Marshall had extremely long series of specimens from both continents and under such circumstances interpretations are bound to differ.

A recent study of *Anodonta* parasites in England (Mitchell and Pitchford, 1953) resulted in large collections of *U. ypsilophora* and *U. intermedia* from England. These collections have now been compared with long series of American *Anodonta* parasites and Wolcott's specimens which are in the Marshall Collection at the Chicago Natural History Museum. I have been unable to find any verifiable record of either *U. ypsilophora* or *U. intermedia* from this country. All collections of *Anodonta* parasites from the new world may be distinguished from the related European species and the distinguishing criteria are discussed below.

Unionicola wolcotti Piersig

Specific diagnosis: With the characteristics of the subgenus *Pentatax*. Body length: male 0.40 to 0.55 mm, female 0.57 to 0.85 mm. Inner margins of coxae weakly sclerotized. No integumentary markings or dorsal plates. Palps normal; setigerous projection placed subterminally on the flexor surface of segment V, projection very broad in female, narrower in male (Figs. 4, 5). All tarsal claws bifid. Leg IV of male heavier than female and provided with modified setae (Fig. 1); lateral surface segment III with 4 elongate setae in a row, segment IV with 2 elongate setae and a dense cluster of 6 to 8 pectinate setae distally placed on the flexor surface, segment V with 9 to 11 pectinate setae in a mass more or less proximally placed on the flexor surface. Leg segment lengths of male given in Table 1.

TABLE 1.—*The average length in millimeters of the segments of leg 4 of the males. The data were taken from 20 specimens from each locality and the range is given in parentheses.*

	Segment 3	Segment 4	Segment 5	Segment 6
U. intermedia (Wollaton, Notts., England)	.204 (.180–.220)	.180 (.170–.200)	.244 (.240–.260)	.194 (.175–.220)
U. wolcotti (Washtenaw Co., Michigan)	.172 (.135–.190)	.153 (.125–.165)	.212 (.170–.230)	.174 (.155–.190)

Hosts: *Anodonta grandis* and *A. cataracta*.
Site of infection: Gill surfaces.
Range: Eastern North America.
Specimens examined: Measurements from a series collected in Four Mile Lake, Washtenaw County, Michigan. Types in the Marshall Collection at the Chicago Natural History Museum.

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The confusion as to the status of this species makes a detailed comparison desirable. *U. wolcotti* is about 7/8th the size of *U. intermedia*. The setigerous projection of the flexor surface of palp segment IV is always subterminal in *U. wolcotti* but is terminal in *U. intermedia*. The leg setae are generally fewer and shorter in *U. wolcotti* than in *U. intermedia*. The two species are clearly distinguished by the length of the leg segments (Table 1). *U. wolcotti* bears 6 to 8 heavy setae in a distal position on the flexor surface of segment IV whereas *U. intermedia* bears from 12 to 16 in the same position. Segment V bears a crowded mass of setae more or less centrally placed on the flexor surface; *U. wolcotti* has 9 to 11 setae in this group, *U. intermedia* bears 15 to 19.

It seems likely that Piersig proposed the variety *wolcotti* on the basis of comparisons between European specimens and the figures of Wolcott (he may have had a few American specimens). The resultant description was not adequate and was quite properly disputed. However, these studies show that *wolcotti* should be recognized as a valid species.

Unionicola formosa Dana and Whelpley

Specific diagnosis: Only the male may be differentiated from *U. ypsilophora*. Body length: male 0.93 to 1.14 mm, female 1.23 to 1.53 mm. Dorsal plate of male large, 0.82 to 1.09 mm long, 0.39 to 0.57 mm wide, margins ill-formed and irregular, with a slight median indentation posteriorly (Fig. 8). Coxae I and II typical, III and IV expanded (Fig. 7). Coxae IV imperceptibly fused with ventral body wall sclerotizations, meso-posterior margins of coxa absent except for a short apodeme at the meso-posterior angle. Sclerotizations of the venter join coxae IV mesally, extends laterally from coxae III and IV up onto the dorsal surface, and extends posteriorly to fuse with the anterior margin of the genital sclerite as well as to enclose the genital sclerite laterally. A narrow, irregular unsclerotized zone often runs posteriorly between coxae IV.

Hosts: Anodonta grandis, A. cataracta, A. marginata, and *A. implicata.*

Site of infection: Gill surfaces.

Specimens examined: Measurements from a series collected in Limestone Lake, 20 miles north of Parry Sound, Ontario.

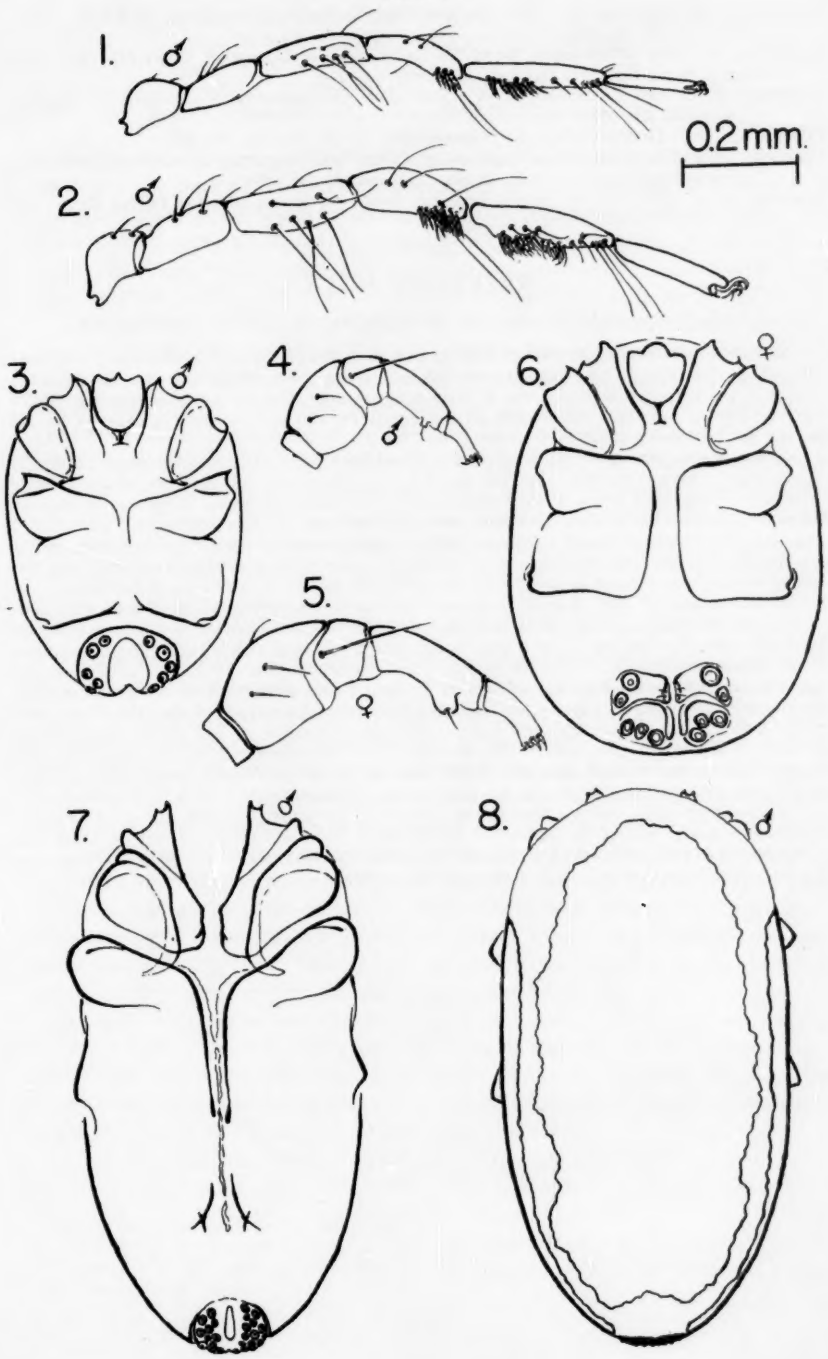
Dana and Whelpley (1836) described *U. formosa* from specimens that were in *Anodonta cataracta* collected near New Haven, Connecticut. While the description is not diagnostic the data do limit the possible identity of the form. Material from *A. cataracta* collected in Massachusetts was examined together with other East Coast collections made by Arthur Humes (now deposited with the Illinois State Natural History Survey) and it is apparent that the only species of the subgenus *Parasitatax* parasitizing *A. cataracta* in the Northeast is the form described above. Although the eastern collections are adequate for identification they are not suited for permanent reference material, so the designation of a neotype must be deferred.

Piersig's name *haldemani* (Piersig, 1900) is a synonym of *U. formosa.*

Explanation of Figures
(The scale applies to Figures 1 and 2.)

FIG. 1. *U. wolcotti,* lateral aspect of leg IV of male.
FIG. 2. *U. intermedia,* lateral aspect of leg IV of male.
FIG. 3. *U. wolcotti,* ventral aspect of male.
FIG. 4. *U. wolcotti,* lateral aspect of male palp.
FIG. 5. *U. wolcotti,* lateral aspect of female palp.
FIG. 6. *U. wolcotti,* ventral aspect of female.
FIG. 7. *U. formosa,* ventral aspect of male.
FIG. 8. *U. formosa,* dorsal aspect of male.

PLATE I



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RESEARCH NOTE

THE SURVIVAL OF SWINE WHIPWORM EGGS IN HOG LOTS

Recent observations by the writer indicate that the eggs of the swine whipworm, *Trichuris suis*, which normally do not hatch outside the host swine, may remain infective for approximately 18 months when kept in water at room temperature. The observations reported in this note were made to obtain information on the infectivity of swine whipworm eggs on an old hog lot in the vicinity of Beltsville, Md.

The test area measured 240 by 40 feet and had been used for swine for many years. It sloped slightly toward the east. In 1949, the last year it was used for pigs, the lot was heavily infested with eggs and larvae of the common species of swine helminths, including whipworms. After lying fallow for 2 years, all fences were removed and the area was graded and planted to grass. The grass produced a firm sod, which still covered the soil at the beginning of the present experiment. The lot was located so that it received no drainage from other hog lots and its surface was exposed to the direct rays of the sun for the greater part of the day.

In the summer of 1955, 3 small temporary enclosures, measuring 40 by 10 feet, were constructed on the long axis of the area, one near the upper end, one near the middle, and one near the lower end. Sixteen pigs, 2 to 4 months old, which had been raised parasite-free according to the method described by Spindler (1942, Proc. Helminth. Soc. **9**: 22–23) were separated into 3 approximately equal groups and put in the pens where they remained for 3 to 4 months. Fecal examinations made prior to the beginning of the experiment, and during the 50-day prepatent period, showed no whipworm eggs. After the expiration of this period, however, all of the test pigs passed the eggs of this parasite. The maximum number of whipworm eggs passed by the experimental pigs was 22,000 eggs per gram (EPG) of feces. The presence of *T. suis* was confirmed in 7 pigs by postmortem examination.

These observations indicate that in the vicinity of Beltsville, Md., the eggs of *T. suis* remained infective to susceptible pigs for at least 6 years in the soil of an old hog lot which was covered with heavy sod for the last half of the period.—CHARLES H. HILL, *Animal Disease and Parasite Research Branch, ARS, Beltsville, Maryland.*

NEMATODE PARASITES OF MAMMALS OF THE GREAT SALT LAKE DESERT OF UTAH*

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During the summers of 1953 and 1954, a study was made of the endoparasites inhabiting the common species of mammals of the region south and west of the Great Salt Lake in the southern arm of the Great Salt Lake Desert in Utah. This is an extensive area which covers several thousand square miles. Most of the collecting, however, was accomplished in a representative area of about 1000 square miles in southern Tooele County, east of the extensive salt flats bordering the lake.

The study was conducted as a phase of the ecological investigation of the native fauna of the area in and about Dugway, Utah. The object of the study was to determine the endoparasites present in native animals, host parasite interrelations, parasite populations, and their distribution in the various habitats present. Dissections of animals and study of the parasites was undertaken both at Dugway and in the Department of Zoology, University of Utah, Salt Lake City. Determination of nematode specimens was made by the author and by personnel from the Animal Disease and Parasite Research Branch of the Bureau of Animal Industry, especially Mrs. M. B. Chitwood. Determination of the mammals was made by the staff of Ecological Research, following Durrant (1952).

ECOLOGICAL DISTRIBUTION

In the region where this study was conducted are several isolated desert mountains and mountain ranges, the slopes of which lead down into broad valleys and extensive flat lands that border the vast salt flats southwest of the Great Salt Lake. Cliffs and exposed rock are characteristic of the higher parts of the mountains. Talus slopes and alluvial fans lead from the mountains into the fine clay soils of the valleys and flats. Sand dunes trace an irregular pattern over flats, valleys, foothills and mountains seemingly in accordance with wind currents.

The tops of the higher mountains are crowned with spruce-fir forests or the highest peaks may rise above timber line into the montane tundra. Below these coniferous forests, the steep mountain slopes usually bear open stands of mountain brush of several kinds that merge with the pigmy forests of juniper on the lower slopes and valley edges. These pigmy conifers extend downward on the slopes several hundred feet lower in altitude in the sand than in other soils.

In some of the higher well drained valleys, the pigmy forests give way to stands of the common sagebrush, *Artemisia tridentata,* if there is sufficient moisture and the soils are free from excessive mineral salts. Below the moisture range of sagebrush, other desert shrubs dominated mainly by shadscale, *Atriplex confertifolia,* extend downward to the edge of the salt flats.

These desert shrubs occupy a belt of scanty rainfall, usually less than 10 inches, and much of the soil is salty or alkaline. Although this area is mainly dominated

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by shadscale, it is associated in different parts of its range with black sagebrush, *Artemisia t. nova;* bud sagebrush, *A. spinescens;* horsebrush, *Tetradymia* spp.; winter fat, *Eurotia lanata;* gray molly, *Kochia vestita;* or greasewood, *Sarcobatus vermiculatus;* each mixture having different community characteristics. In some areas, the greasewood occurs in pure stands. In some cases, sand dunes are unvegetated and free to blow with the wind; in other cases, the sand is partly or completely held by the vegetation and prevented from blowing. The vegetation shows various stages of succession from the bare dunes to complete stabilization. Around the edge of the salt flats is a belt of pickleweed.

The barren salt flats and drifting sand dunes are devoid of permanent animal life, although animals may occasionally cross them. Mammals living in the desert occupy one or more of these types of vegetation. The number of species of mammals occupying these vegetation types gradually increases from one in the pickleweed upward to several in the foothills, with the exception of the vegetated dunes which are approximately as productive of rodents and rabbits as the foothills, several hundred feet higher in altitude.

The mammals covered in this study are mainly rodents and rabbits; these serve as hosts to the nematodes, thus the distribution of these mammals is of primary significance in studying the distribution of the nematode parasites. Indirectly, therefore, the vegetation may be of primary importance in determining the distribution of many of the nematodes by influencing the distribution of their hosts.

COLLECTION OF MATERIAL

Mammal specimens were collected almost entirely by means of live traps and were sacrificed for this and other studies. Exceptions were the rabbits and larger carnivores, which were shot. Dissections were made of the internal organs of the freshly killed animals when possible, but if time did not permit, the viscera were preserved in 10% formalin within a few minutes following death, thus ensuring suitable preservation of the parasitic material. Dissections of the tracts of the larger animals, such as coyotes and rabbits, were made following commonly used procedures. Tracts from the smaller forms were flooded with physiological saline solution in an open petri dish and examined with the aid of a dissecting microscope.

Living nematodes were killed in a relaxed state by heating the saline gradually to 120° F. Specimens were fixed in Kahle's solution overnight and stored in either 4% formalin or 70% alcohol. Permanent mounts for study were made, using a technique developed by the author (Grundmann 1955).

RESULTS

Twenty-three species of nematodes were recovered during the study. Of these, 6 are under consideration as probable new species by Mrs. M. B. Chitwood. Although the complete carcasses were given gross examination, only in the case of the desert wood rat, *Neotoma lepida,* were worms obtained from a site other than the digestive system. In this case, numerous larval ascarids were taken from the livers of a number of specimens. In each instance, the liver also contained large numbers of cysticerci of the tapeworm, *Paruterina candelabraria* Goeze 1782, thus indicating that the source of infection for both nematode and tapeworm is probably the same.

The following list of species contains the nematode parasites obtained. Pertinent data include incidence of infection, site in the host and habitat of each host species.

TRICHURIDAE

Trichuris sp. (possibly *T. perognatha* Chandler 1945)

Host: Perognathus formosus incolatus Hall, long tailed pocket mouse. *Location:* Cecum. *Incidence:* 2 of 35 specimens, 6%. *Habitat of Host:* Mixed brush of foothill and mountain slopes.

The nematode specimens differ from Chandler's species in the absence of a vaginal protrusion. Eight male and 8 female specimens were recovered.

Trichuris dipodomys Read

Hosts: Dipodomys ordii, Ord kangaroo rat, and *D. microps,* chisel-toothed kangaroo rat. *Location:* Cecum and anterior large intestine. *Incidence: D. ordii,* 11 of 99 specimens, 11%; *D. microps,* 24 of 41 specimens, 58.5%. *Habitat of Host: D. ordii* reaches its greatest concentration in the vegetated dunes and the sandy areas of the juniper-brush cover. *D. microps* inhabits the outer edge of the sandy areas, mainly in the mixed brush of the foothills, shadscale-gray molly-greasewood and shadscale-bud sage habitats in the flatlands.

The worms occur in the animals through the periods studied (summer) with no apparent change in incidence. Distribution throughout the range of the host appears to be irregular. When infection was noted in the specimens from one locality, it was generally present in the majority of animals collected at that place. In other localities, no infected animals were taken. Localization or isolation of habitat and the sedentary habits of individuals help in understanding this phenomenon. When infection in the host was light, the worms were restricted to the cecum, usually toward the blind end; if 20 or more worms were present, they usually overflowed the cecum into the anterior end of the large intestine.

ASCARIDAE

Toxascaris leonina (Linstow 1902)

Host: Canis latrans lestes Merriam, coyote. *Location:* Small intestine. *Habitat of host:* The coyote ranges over the entire area.

Two coyotes were examined; this parasite was recovered from both. A previous study in Utah (Butler and Grundmann 1954) has shown this species to be the common ascarid of the coyote.

Ascaris sp. (larval)

Host: Neotoma lepida lepida Thomas, desert wood rat. *Location:* Liver. *Incidence:* 6 of 31 animals, 19%. *Habitat of host:* Foothill and mountain areas. Prefers rocky situations in the juniper-brush communities.

In specimens where this form was present, the livers possessed large numbers of the larval ascarids. The larvae were not encysted, and in most cases not coiled, and demonstrated a limited ability to move through the tissue. The majority of the larvae were near the surface and immediately below the serosal covering. All such ascarid infections were accompanied by numerous cysticerci of *Paruterina candelabraria* Goeze 1782, a tapeworm reaching its adult stage in the owl. The constant occurrence of the larval stages of both together indicates that the source of infection for the wood rat is the same for both parasites and that the definitive host for both in this region is probably the horned owl, *Bubo virginianus,* a common inhabitant of the same habitat as the wood rat. Examination of regurgitated pellets from owls in the study area has produced skulls of the wood rat. Ascarid larvae of species inhabiting carnivores and using rodents as intermediate hosts have been previously reported and discussed by Tiner (1951 and 1953).

OXYURIDAE

Aspicularis tetraptera Schultz 1924

Host: Peromyscus maniculatus sonoriensis (LeConte), deer mouse. *Location:* Anterior large intestine. *Incidence:* 2 out of 115, 2%. *Habitat of host:* The deer mouse has been found in nearly all of the habitats available in the study area, although it is more numerous in the junipers and brush habitats of the foothills and in the shadscale-gray molly-greasewood vegetation.

This parasite was recovered only in the case of laboratory-reared mice. The infection was obviously the result of contamination from white laboratory-reared mice residing in the same building.

Aspicularis sp.

Host: Desert wood rat. *Location:* Anterior large intestine. *Incidence:* 5 of 31 specimens, 16%.

This form appeared in the host during early August and a majority of the specimens examined possessed it at that time. This nematode is being described by Mrs. M. B. Chitwood as a new species.

Dermatoxys velligera (Rudolphi 1819)

Host: Lepus californicus deserticola Mearns, black-tailed jack rabbit. *Location:* Cecum. *Incidence:* 3 of 19 rabbits, 15%. *Habitat of host:* Found in all habitats except the pickleweed and the higher mountains.

This parasite is widely distributed in the wild rabbits; it has been found in jack rabbits and cottontails in a number of other areas of Utah.

Heteroxynema cucullatum Hall 1916

Host: Eutamias dorsalis utahensis Merriam, cliff chipmunk. *Location:* Cecum and large intestine. *Incidence:* 9 of 18 animals, 50%. *Habitat of host:* The host is found only in the brush areas of the mountains. Preferred nesting sites are on steep, rocky slopes.

This species was recovered from hosts collected in the Stansbury Mountains. It also was found in the majority of a series of specimens of the Say chipmunk, *E. quadrivittatus,* collected at Dewey, in eastern Utah, but not from the least chipmunk *E. minimus,* in the study area.

Oxyuris sp.

Host: Eutamias minimus pictus (Allen), least chipmunk. *Location:* Cecum. *Incidence:* 2 of 9 specimens, 22%. *Habitat of host:* Greatest numbers are found in the vegetated dunes of the flatlands. Also occasionally found in the shadscale-gray molly-greasewood vegetation and the brush of the lower foothills. Prefers areas of tall shrubs, grasses and herbaceous plants.

Citellina triradiata Hall 1916

Host: Citellus leucurus leucurus Merriam, antelope ground squirrel. *Location:* Cecum. *Incidence:* 52 of 64 specimens, 82%. *Habitat of host:* Usually found in the junipers and brush of the foothills, occasionally in the greasewoods. It prefers sandy or loamy soils in uneven terrain with burrows in side hills or banks.

This parasite was found in animals from all localities in the host range and may be considered to be the most prevalent parasite of this host. The fully gravid females appear to migrate from the cecum and in heavy infections may be found throughout the large intestine. The usual parasite population recovered contained specimens in all states of development indicating that infection is probably more or less continuous during the period of the year covered by the study.

Passalurus nonannulatus Skinker 1931

Host: Black-tailed jack rabbit. *Location:* Cecum. *Incidence:* 5 of 19 rabbits, 26%.

A common parasite of wild rabbits that appears to be present throughout the year; it was recovered from rabbits taken in January as well as those examined during the summer months.

Syphacia eutamii Tiner 1948

Host: Cliff chipmunk. *Location:* Cecum. *Incidence:* 13 of 18 specimens, 72%.

All hosts studied were collected from sites in the Stansbury Mountains. Infection appeared in specimens from each site, indicating that this parasite probably is common. The least chipmunk, *Eutamias minimus,* which occupies a different habitat in the foothills and vegetated dunes, was not found to harbor this parasite, although it was recovered by the author from a series of the Say chipmunk, *E. quadrivittatus,* collected along the Colorado River near Dewey, Grand County, Utah.

Syphacia samorodini Erickson 1938

Hosts: Reithrodontomys megalotis (Baird) western harvest mouse, and deer mouse. *Location:* Cecum. *Incidence:* 10 of 44 in the harvest mouse, 22%. 15 of 115 in the deer mouse, 13%. *Habitat of Host:* The harvest mouse is usually found in the vegetated dunes and the brush habitats of the foothills. It prefers locations with grass and herbaceous plants.

This parasite was found in host specimens from two localities only; one along the sewer line in central Dugway valley; the other in the Stansbury Mountains.

Wellcomia evoluta (Linstow 1899)

Host: Erethizon dorsatum epixanthum Brandt, porcupine. *Location:* Illiocecal region. *Habitat of host:* The porcupine is widely distributed in the mountains of Utah and may be encountered as low as brush habitats of the foothills. About 150 specimens were recovered from a single porcupine.

TRICHOSTRONGYLIDAE

Heligmosomum sp.

Host: Ord kangaroo rat. *Location:* Duodenum. *Incidence:* 4 of 99 specimens, 4%.

In all host specimens containing this parasite the intestinal tract contained quantities of seed coats and insect parts mainly of coleopteran origin. The duodenum was also enlarged.

Molineus felineus Cameron 1923

Host: Taxidea taxus taxus (Schreber), badger. *Location:* Duodenum and jejunum. *Habitat of host:* Found in areas of heavy rodent populations.

Approximately 150 specimens, about equally divided between the sexes, were recovered from one of the 2 animals examined. Anterior ends of the specimens were firmly embedded between the intestinal villi; the posterior ends extended free into the intestinal lumen.

Nematodirus leporis Chandler 1924

Host: Black-tailed jack rabbit. *Location:* Ileum. *Incidence:* 2 of 19 rabbits, 10.5%.

Each of the 2 rabbits contained from 300 to 500 parasites, of which sexes were about equal in numbers; many pairs were attached together.

Nematodirus neotomae Hall 1916

Host: Desert wood rat. *Location:* Duodenum and jejunum. *Incidence:* 10 of 31 specimens, 32.2%.

The species was present during late summer and autumn in specimens from all localities sampled, including the Stansbury Mountains to the northeast of Dugway.

PHYSALOPTERIDAE

Physaloptera sp.

Host: Antelope ground squirrel. *Location:* Stomach. *Incidence:* 2 of 64 specimens, 3%.

Several specimens from Little Granite Mountain, Tooele County, Utah were found to possess several females worms in which the uterine characters differed from *P. massino.* No males were recovered.

Physaloptera massino Schultz 1927

Host: Antelope ground squirrel. *Location:* Stomach. *Incidence:* 5 of 24 specimens, 21%.

All host specimens containing this nematode were collected during a period extending from 1 June to 7 July, 1954, from Little Granite Mountain. *Citellus* collected from the same areas later did not possess the parasite. It probably utilizes one or more of the numerous species of beetles as intermediate hosts.

THELAZIIDAE

Protospirura numidica Seurat 1914

Hosts: Ord kangaroo rat; western harvest mouse; *Peromyscus crinitus pergracilis;* canyon mouse; *P. truei nevadensis,* pinyon mouse; deer mouse, and *Onychomys leucogaster,* grasshopper mouse. *Location:* Stomach and esophagus. *Incidence:* 2 of 99 Ord kangaroo rats, 2%. 23 of 44 western harvest mice, 52%; 8 of 19 canyon mice, 41%; 66 of 115 deer mice, 57%; 4 of 17 pinyon mice, 23%; 2 of 5 grasshopper mice, 40%. *Habitat of hosts:* The hosts collectively come from all habitats of the study area. The greatest populations occur in the vegetated sand dunes and the juniper-brush habitats of the foothills and mountains.

Immature adults began to appear in the stomachs of hosts during the last week in July, and completely gravid females were present several weeks later. Immature forms were generally found confined to the stomach except in cases of heavy infection when they might be found with fully matured adults in the lower esophagus.

The parasite appears to be widely distributed and was obtained from specimens taken in all localities collected; it seems to be almost restricted to species of the rodent Family Cricetidae. In some habitats, cricetid rodents live in association with members of the Families Geomyidae

and Heteromyidae without these species becoming parasitized, except for a limited infection in *D. ordii.*

A common habit possessed by all of the host species was the ingestion of insects as food. Stomach and cecal contents commonly were found to contain recognizable fragments of insects, usually of Coleopteran (beetle) origin. It is also well known that many spiruroid worms utilize beetles as intermediate hosts.

Rictularia coloradoensis Hall 1916

Host: Antelope ground squirrel. *Location:* Duodenum. *Incidence:* 2 of 64 specimens, 3%.

Rictularia sp.

Host: Deer mouse. *Location:* Duodenum. *Incidence:* 3 of 115 specimens, 3%.

This is probably a new species. It was recovered from 3 animals. All infections consisted of 1 or 2 worms; no males were recovered.

DISCUSSION

During the course of the investigation, 537 mammals of 21 species were examined. These animals ranged in size from small rodents such as *Perognathus, Microdipodops,* and *Reithrodontomys,* to the larger carnivores such as the coyote and bobcat. Although the number of each species dissected varied somewhat with the species abundance and the ease of capture, it is felt that the sample of most species was adequate to provide significant results. In the case of several mammal species, such as the larger carnivores, numbers examined are too small to be satisfactory indicators. Unquestionably the dissection of more specimens will provide additional species of parasites.

Animals of all species collected during the spring and early summer (May, June and early July) showed a combined prevalence of infection of 39.2%. Animals of the same species, and in approximately the same numbers, collected between July 15 and the end of September showed a combined prevalence of 60%, or an increase of approximately 50%. This increase in incidence, although variable among the species involved, was noted in all except the Ord kangaroo rat, in which the late season group showed a decreased prevalence. In the several species of Peromyscus, the increase in incidence approached 100% in the late season group.

This increase in incidence during July, August and September is considered to be induced by several causes, chief among which is the occurrence of one species, *Protospirura numidica* Seurat 1914, which was present in the stomachs of 6 species of rodents and began to appear as immature worms during the latter part of July. The incidence of infection with this parasite increased to a point where almost all specimens taken in infected localities were parasitized. Several species of *Physaloptera* inhabiting the stomach of the antelope ground squirrel are also in this category.

There appeared to be an increased use of insects as food at the time of the increased incidence of infection in late summer and autumn. This trend was noted in most of the host species studied. Although insects seem to be widely used by many rodents, some species, such as the black-tailed jackrabbit and the chisel-toothed kangaroo rat are herbivorous and rarely are insect parts encountered in the digestive tract. The Ord kangaroo rat, however, utilized more insects in the diet, occasionally being found with the intestine and caecum largely filled with chitin fragments. Specimens of the latter group usually are found parasitized with *Protospirura.*

Population density of hosts is important in determining distribution of the parasites; areas of dense population have higher indices of parasitism. Distribution of

infection appears to be facilitated by greater activity and wider distribution of the host. Where the host has a wide ecological distribution, parasitic species appear to be more or less uniformly distributed; a given parasite is usually recovered from hosts taken on all parts of the range. Higher rates of infection usually come from areas of high population density. Hosts with more restricted distribution or with more restricted activity may have an irregular distribution of parasites. For example, in the chisel-toothed kangaroo rat, the species of *Trichuris* inhabiting the cecum has been found in a high proportion of specimens from one locality and none from another. These conditions were found to be especially true in desert mountains separated by geographic barriers that inhibit migration.

In general it was noted that the species of rodents that had the greatest ecological distribution in the area had the largest number of species of parasites. The white-footed deer mouse, found in all habitats, was parasitized by 6 species of nematodes. The antelope ground squirrel, next widest in habitat distribution, was parasitized by 4 species. The wide-ranging jack rabbit possessed 3 species. One exception was found in the chisel-toothed kangaroo rat, the third most widely distributed species, which was parasitized by only a single species of *Trichuris*. However, 58.5% of all specimens collected possessed 1 or more whipworms in the cecum.

Wider ecological distribution with the resulting greater variety of foods utilized are important factors in determining the overall incidence of infection and numbers of species affecting the host. Rodents, such as the widely distributed deer mouse and the antelope ground squirrel have a variable diet which may partly account for the large number of species parasitizing them. The Ord kangaroo rat, which includes vegetation, seeds and insects in its diet, had 3 species of nematode parasites, whereas the chisel-toothed kangaroo rat, which has a diet almost exclusively of vegetation, was found to possess but 1 species with a 58.5% of infection.

SUMMARY

A study was conducted of the nematodes inhabiting mammalian hosts of 22 species in the Great Salt Lake Desert Region west and south of the Great Salt Lake in Tooele County, Utah. Emphasis in the study was placed not only upon a survey of the species present, but also upon habitat and host distribution, parasite population and incidence of host infection, seasonal distribution and interrelationships of hosts.

A total of 527 animals were examined. Autopsies were performed on freshly killed animals obtained by means of live-trapping in the field. Sufficient numbers of specimens were examined in most of the rodent species to provide indicative results. In several species, notably carnivores, numbers examined were small. The high density of rodent populations, especially in the vegetated sand dunes and the juniper and mixed brush habitats of the foothills and mountain slopes are of special significance in the study because of their greater potential for parasite transmission among the hosts.

Prevalence of infection with nematodes of all mammals examined during May, June and early July was calculated at 39.2%. Approximately the same number of animals collected in late July, August and September showed a prevalence of approximately 60% or an increase of about 50%. The increase was found to be due to (1) seasonal distribution of species, such as *Protospirura numidica* Seurat 1914,

and several species of *Physaloptera* not present early in the season, (2) the increased use of insects as food (this increases the incidence of infection with nematodes that utilize insects as intermediate hosts), (3) increased density of population of hosts, which usually is greater in late summer.

In general, species of rodents having the widest distribution in the area were found not only to harbor the largest number of parasitic species, but also had the highest percentage of infection. Diversity of habitat and food available in the several habitats may also be important factors.

Twenty-three species of nematodes were recovered, of which 6 are under consideration as probably undescribed species. The species found are distributed through the orders Enoplida, Rhabditida and Spirurida; and the families of Trichuridae, Ascaridae, Oxyuridae, Trichostrongilidae, Physalopteridae and Thelaziidae.

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RESEARCH NOTES

A NOTE ON THE *IN VITRO* DEVELOPMENT OF *NIPPOSTRONGYLUS MURIS*

During an investigation of the respiratory physiology of *Nippostrongylus muris* larvae, the following incidental observation was made upon the *in vitro* survival and development of the parasitic larvae of this parasite.

Third-stage larvae, isolated by peptic digestion from the lungs of experimentally infected rats 48 hours post-infection, were observed in different media. These larvae were late third-stage; in some the sheaths had just begun to loosen in preparation for the first parasitic ecdysis (corresponding to Fig. 2, Plate I of Weinstein and Jones, 1956, J. Parasitol. **42:** 215–236).

Larvae placed in cotton-plugged 15 ml. centrifuge tubes containing approximately 3 ml normal rat serum (no aseptic precautions observed) survived actively for at least 8 days at room temperature (at which time the bacterial and mold contamination was pronounced and larvae became sluggish and died). These larvae grew considerably in length and molted to the fourth stage. Before death they showed the typical anterior cuticular expansion characteristic of this stage, and a bursal development in the males intermediate between that of Fig. 4, Plate I and Fig. 5, Plate II of Weinstein and Jones (cited above).

Similar larvae placed in immune rat serum developed typical precipitates about the natural openings and survived for only 2–3 days, during which period no development was observed.—CALVIN W. SCHWABE, *Department of Parasitology, School of Medicine, American University of Beirut, Beirut, Lebanon.*

THE PREVALENCE OF *EURYTREMA PROCYONIS* DENTON (TREMATODA: DICROCOELIIDAE) IN SOME MAMMALS FROM MARYLAND

Eurytrema procyonis was first reported from raccoons, *Procyon lotor,* in Texas (Denton, J. F., 1942, Proc. Helm. Soc. Wash. **9**: 29–30). It has been reported in raccoons from Connecticut (Penner, L. R. *et al,* 1954, Proc. Helm. Soc. Wash. **21**: 34–35) and from a red fox, *Vulpes fulva,* in New York (Stunkard, H. W., 1947, J. Parasitol. **33**: 459–466). Although Stunkard originally identified the parasite as *E. vulpis,* n. sp. *provis.,* he subsequently reduced it to synonomy with *E. procyonis* (Stunkard, H. W. and L. J. Goss, 1950, J. Parasitol. **36**: 574–581). The adult flukes occur in the pancreatic ducts of these hosts.

During studies conducted by the authors, trematodes considered to be morphologically identical with *E. procyonis* Denton, have been observed in pancreatic tissues from both raccoons and foxes. These parasites first came to our attention in sectioned material being studied in connection with distemper investigations (Kilham, L. *et al,* 1956, Am. J. Vet. Res. **17**: 144–148). Subsequently the pancreas of all raccoon and fox presented for autopsy were examined in fresh condition for evidence of this infection. From one to thousands of flukes per infected animals were removed from the pancreatic ducts.

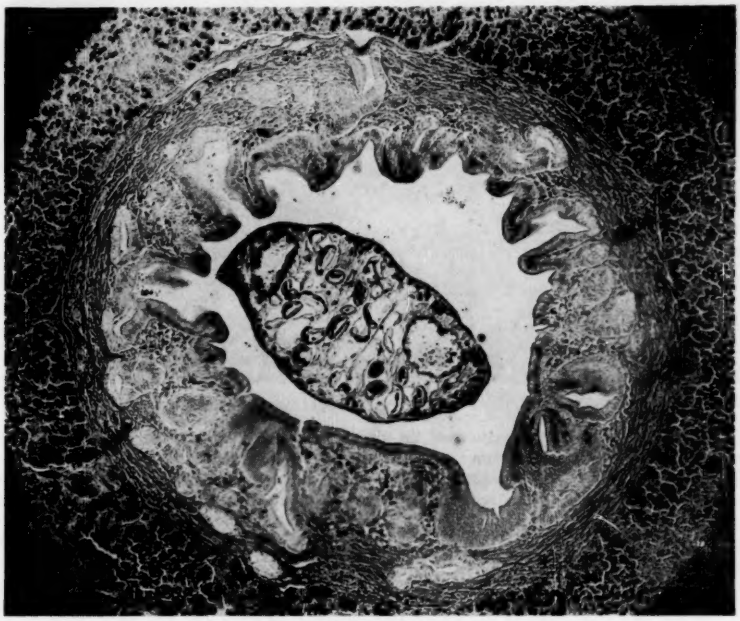


FIG. 1. *E. procyonis* in distended pancreatic duct of a raccoon. ×87.

The majority of the animals were trapped at the Patuxent Research Refuge near Laurel, in Prince Georges County. Raccoons from other areas in Maryland were collected in a sick condition, suspected of rabies infection. In all cases, distemper was demonstrated. Of 5 raccoons examined from Wicomico County on the Eastern Shore, 4 were infected with *E. procyonis.* Individual raccoons from Burtonsville, Montgomery County, and Cokesbury, Somerset County, were also infected. Although some of these raccoons were heavily infected with *E. procyonis,* there was no evidence that this was a contributing factor to their apparent illness. Several of the raccoons trapped at the Patuxent Refuge were heavily parasitized with this pancreatic fluke but evidenced no outward signs of malaise. Infected animals were collected in every month of the year.

E. procyonis was recovered from 17 of 37, or 46%, of raccoons examined. Eleven of 27, or 41%, of apparently healthy raccoons trapped on the Refuge were infected. These 27 ani-

mals represent approximately one-fourth of the total estimated population of raccoons on the Refuge. The parasites were also found in 2 of 3 gray foxes (*Urocyon cinereoargenteus*). None were found in the pancreas of 8 red foxes. One infected gray fox was a rabies suspect from Mechanicsville, Montgomery County; the other two, and all red foxes, were from the Patuxent Refuge.

Similar pathology was observed in raccoons and foxes. In heavily infected animals the pancreas was pale yellowish pink, soft and spongy, and enlarged.

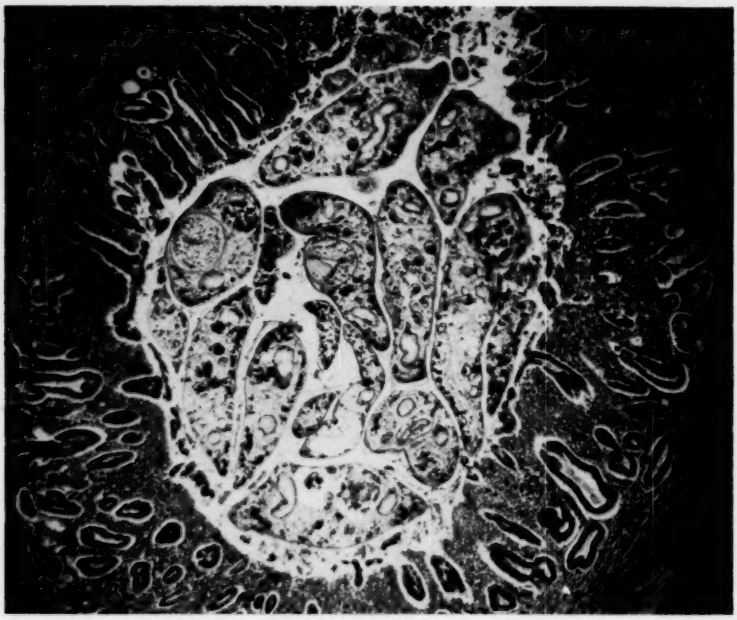


FIG. 2. Numerous *E. procyonis* in distended pancreatic duct of a gray fox. ×50.

The histopathological examination of the pancreas of the raccoon and fox showed numerous *Eurytrema procyonis* in the pancreatic ducts. In Figure 1 a mature fluke is shown in one of the small pancreatic ducts of a raccoon. Figure 2 illustrates a number of flukes in an interlobular duct of a fox. It will be noted that in each of the sections, the ducts appear distended, but there is no injury to the epithelial cells, and no fibrosis or cellular infiltration. In some of the sections of the pancreatic ducts, flukes were seen to have a small bit of the epithelial lining in their suckers, but there was no evidence of thickening or piling up of the columnar epithelial cells. The islets of Langerhans appeared normal in all the animals examined; however, it was reported by Penner *et al* (1954) that the urine from some of the raccoons in their studies was cloudy, had a pH of 5.6, and gave a positive test for acetone, albumin, and sugar. —CARLTON M. HERMAN, *Patuxent Research Refuge, U. S. Fish and Wildlife Service, Laurel, Md.*; PRESTON M. BAUMAN, *W.R.A.I.R., Walter Reed Army Medical Center, Washington, D. C.*; AND ROBERT T. HABERMANN, *National Institutes of Health, U. S. Public Health Service, Bethesda, Md.*

SURFACE-WINTERING OF AQUATIC SNAILS IN CENTRAL WASHINGTON

Four species of gastropods occur commonly in the irrigation canals of the Columbia Basin Project. These are: *Physa propinqua; Gyraulus similaris; Fossaria perplexa; Stagnicola palustris nuttalliana.* During the late summer and fall of 1955, large populations of these snails were noted and examined after irrigation water had been shut off. The Moses Lake Weather Bureau station, located in the study area, supplied data showing that the 5 months of October 1955 to February 1956 were the coldest since irrigation began in 1952. The mean of the average temperatures was 30° F, 6 degrees colder than the equivalent means of the preceding 3 winters. The maximum temperature was 71° F in October, the minimum, −17° F in February: there

were 12 periods ranging in length from 1 to 16 days, and totaling 55, in which the maximum temperature was 32° or less. Snow was recorded in more than trace amounts for 87 days; during the coldest part of the winter, 3 inches were present.

Individuals of the four species over-wintered in the usual habitats—partly or wholly buried in mud, under stones, in crevices of the canal bottoms, under vegetation, in weir boxes, etc. However, the 2 lymnaeids, *S. palustris nuttalliana* and *F. perplexa*, survived on the surface of canal banks, unprotected except for transient snow cover. Survivors were found in lateral canals ranging from 3 to 6 feet in depth and 6 to 20 feet in width.

When the canals were checked in the autumn, hundreds of living and dead specimens of both lymnaeids were found distributed from the high-water line to the bottom of the canals. In most of the living snails the aperture was applied to the moist substrate and closed by a well-developed epiphragm.

S. palustris nuttalliana, though often near the surface of full canals, rarely left the water. In drained canals, it occurred in large numbers on the bottom and on the lower sides. Fewer were found on the higher portions of the canal sides. Specimens at the latter site had failed to follow the receding water. *F. perplexa*, being amphibious, occurred in greater numbers at, near, and slightly above the high-water mark.

East-west canals differed from north-south canals studied in March, April and May in that they showed a marked contrast in numbers of survivors on the opposite banks. No living specimens were found on dry, nearly barren southern exposures. The upper parts of the north-facing slopes were principally covered by a moist layer of algae and moss. More survivors were recovered from this habitat than from the drier bottom of the canal. The number of survivors on the surfaces of the north-south canals was about the same on both banks and was comparable to that on the north-facing slopes of the east-west canals.

Field-collected gastropods were divided into 2 groups. In the first, the number of survivors was determined by placing them in water at room temperature. Individuals not crawling within 24 hours were discarded. Of 127 *S. palustris nuttalliana* taken from exposed surfaces of empty canals, 34% had survived. Of 152 *F. perplexa* collected dry from both exposed and protected sites, about 6% had survived.

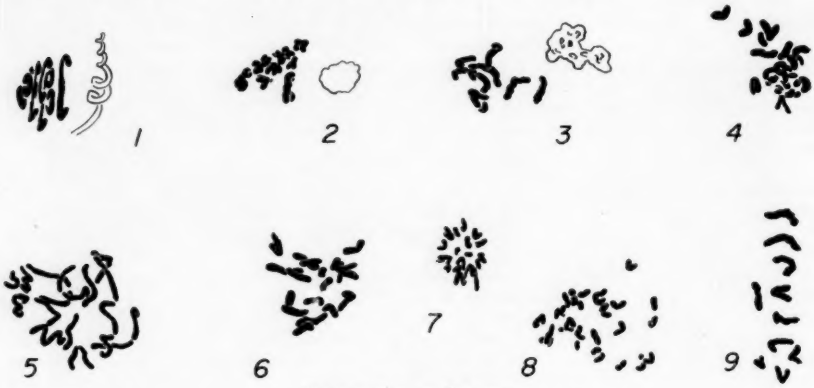
Individuals of the second group were subjected dry to consecutive 24-hour periods at 38, 23, and 38 degrees Fahrenheit respectively, then water at room temperature. Of 44 *S. palustris nuttalliana* which had epiphragms, 25% withstood freezing when maintained dry, as when collected. Of 30 *F. perplexa* which had epiphragms, 2 survived similar treatment, but all 10 which were active when found in melted ice died when re-frozen in water. More gradual chilling probably would have resulted in greater survival.

Olivier (1956, J. Parasitol. **42**: 84) reported that 2 Brazilian snails can survive the dry season in the protection of vegetation at ground level. He concluded that the snails apparently did not enter the soil to avoid the consequences of drying. Observations on the 2 lymnaeids in the Columbia Basin of Washington indicate that they do not need to penetrate the soil to survive for as long as 6 months in dry canals. In addition they were able to withstand temperatures considerably below freezing without protection other than snow. (This investigation was supported in part by research grant E900 from the National Microbiological Institute, of the National Institutes of Health, Public Health Service, and in part by funds provided for biological and medical research by the State of Washington Initiative No. 171.)—CHARLES W. MCNEIL AND WALDEMAR M. WALTER, *Department of Zoology, State College of Washington; Pullman, Washington.*

THE CHROMOSOMES OF *TAENIARHYNCHUS SAGINATUS* (=*TAENIA SAGINATA*) GOEZE, 1782

During a study (supported in part by the U. S. Atomic Energy Commission Contract No. AT(40-1) 1749) of the effect of radiation upon cestodes, we examined the chromosomes of a specimen of the "beef tapeworm," obtained from a patient at Fort Sanders Presbyterian Hospital, Knoxville, through the kindness of Mr. Richard Lee. The specimen had been passed after saline purge following atabrine therapy. It consisted of 2 pieces, without the scolex, totalling about 15 feet in length, with young as well as gravid proglottids. The specimen was very active in warm saline, but had become bright yellow from the drug. Selected proglottids were finely minced in Carnoy's (6 pts. alcohol, 1 part chloroform, 1 part glacial acetic acid). After 12 hours' fixation, these were hardened for several days in 95% alcohol, after which aceto-orcein squashes were made of dissected testes and parts of the young uterus. Because only very few portions seemed to be well fixed, many slides had to be discarded. The chromosomes of selected cells were drawn at the same magnification with the aid of a camera lucida, under N.A. 1.30 objective with oil on the condenser. Unfortunately only the oocytes and young embryos provided good examples of meioses and mitoses; study of spermatogenesis must await better material. The chromosomes number 20, diploid. A reliable idiogram could not be de-

rived, but there appear to be (Fig. 9) 4 large "V's", 2 (possibly 3) large "J's" and 3 small "V's", in the haploid set of 10. The range in size, in first cleavage metaphase, is from 3 to 5 microns. Although the number is the same as that reported by Jones and Ciordia (1956, J. Parasitol. **42:** 207) for *Taenia pisiformis,* the morphology is not similar.



DESCRIPTION OF FIGURES

FIGURES 1, 2. First and second metaphase of meiosis in the oocyte, showing sperm head and male pronucleus in outline.

FIGURE 3. Haploid set of chromosomes (maternal) with male pronucleus in prophase.

FIGURE 4. Prophase of first cleavage, showing 10 distinct maternal chromosomes and confused mass of paternal chromosomes.

FIGURES 5, 6. First cleavage, prophase and metaphase.

FIGURE 7. Metaphase, fourth or fifth cleavage.

FIGURE 8. Metaphase, polyploid or aneuploid nucleus from early embryophore (*ca.* 26 chromosomes).

FIGURE 9. Separate chromosomes of a haploid nucleus.

It is interesting that the developing embryophore of the older embryos contains many polyploid nuclei, some of which were observed in a stage of division (Fig. 8). Further study may reveal the role played by these giant nuclei in the formation of the characteristic striated embryophore of the fully developed embryo.—ARTHUR W. JONES AND KATHLEEN D. WYANT, *Department of Zoology and Entomology, The University of Tennessee, Knoxville.*

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ERRATA

Page 563, Volume 42, number 6. The figures were incorrectly numbered. Figure 1 should be Figure 4. Figure 2 should be Figure 3. Figure 3 should be Figure 2. Figure 4 should be Figure 1.

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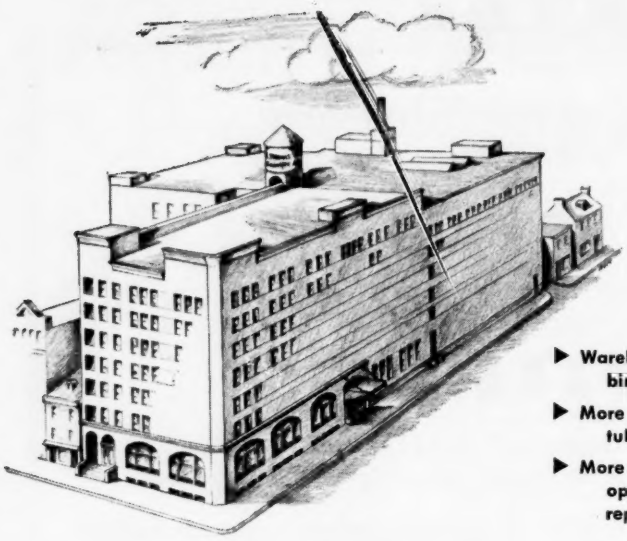

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